ENDOCRINE STUDIES

XLIII. THE GASEOUS METABOLISM OF SOME DWARFS AND GIANTS

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FIVE FIGURES

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During the visit of a circus troupe to Boston, through the courtesy of the several members of the group, the writer had the unusual opportunity of measuring the respiratory metabolism of a group of dwarfs of adult years. In addition, a number of the other members of the company, an acromegalic giant, a 'fat lady,' two alleged albino negroes, and a South African bushman, all contributed a number of measurements. In the case of the giant, J.E., a fairly complete objective study was made, the results of which have been reported elsewhere (Rowe and Mortimer, '34). Extensive radiographic studies were made of all of the above and the results are shortly to be reported by one of my colleagues. The present paper will be limited to the results of the respiratory metabolism study in which five dwarfs, the giant and the 'fat lady' took part. All of these represented different types of abnormal pituitary function; for purposes of contrast and extension, I have included the data from three other patients who in earlier years were completely studied in connection with other investigations. One of these was a massively obese boy, the other two, women with marked retardation of their physical development. For purposes of orientation, the significant physical measurements of the several members of the group are collected in tabular form.

TABLE 1
Physical measurements

NO.	NAME	SEX	AGE (YRS.)	HEIGHT (CM.)	WEIGHT (KG.)	AREA (SQ.M.)	SITTING HEIGHT INDEX
1	L.G.	F	22	95.0	16.3	0.639	0.566
2	T.E.	F	18	107.3	19.5	0.753	0.537
3	G.E.	F	34	113.6	21.8	0.823	0.511
4	D.E.	F	26	125.8	28.4	0.992	0.523
5	M.H.[1]	F	21	140.0	43.9	1.289	0.530
6	A.H.[1]	F	17	141.7	33.4	1.158	0.550
7	H.E.	M	31	107.7	21.1	0.781	0.544
8	B.G.[1]	M	14	147.2	189.5	2.489	0.603
9	R.P.	F	24	160.3	182.1	2.604	0.563
10	J.E.	M	26	228.6	163.3	3.125	0.483

[1] The interpolated cases.

The first four in the series as well as no. 7 are professional dwarfs, no. 9 was the fat lady and 10, the giant. The several figures speak for themselves; only the sitting height indices (i.e., the total divided by the sitting height) call for any comment. My own averages for groups of several thousand men and women of relatively normal configuration are 0.524 for the male and 0.533 for the female. These values are closely in accord with those of Pfitzner ('22) who reports the same value for the male and 0.528 for the female, together with yet other values from similar sources. Recognizing that these are average values only, defining a median in the zone of the normal, a tolerance of $\pm$ 0.020 may be conceded as permissible limits of variation within the convention. Extreme values from my own series (unpublished data) are respectively 0.648 in the male and 0.656 in the female, these being achondroplastic dwarfs while the antitheses are presented in so-called 'eunuchoid' types of pituitary disease with 0.476 and 0.483, respectively. Reverting to the present series, nos. 1, the smallest dwarf, and 9, the fat lady, show high indices, while nos. 2, 3, 4 and 7, all members of one family, fall within the conventional normal zone. The fat boy, no. 8, with the very high index, is an artefact as his huge weight operating since birth had produced marked deformity of the lower limbs. For this reason his total height was not repre-

sentative as it made no allowance for the bowing of the legs. The proportions of the giant are characteristic of this condition in which the longitudinal growth is most marked in the long bones.

A brief comment on each member of the series may aid in further orientation and assist in the interpretation of the values measured for the individual energy exchange.

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No. 1, L.G., is the youngest of three daughters, of whom the oldest, A., age 24, is also a dwarf but has the further handicap of a retarded mentality. The second girl, Lo., is 23 years old, of normal size, and entirely normal in all other respects. The subject of this study, L., was normal at birth but failed to grow so that "at the age of 6 years she could walk under a table without bending." Her teeth were very late in appearing, and she had never matured. The oldest sister, A., has menstruated a few times. The parents are people of normal size and the family history is in no sense remarkable.

Nos. 2, 3, 4 and 7, as already noted, are all members of the same family. In addition to these four, their parents had four more daughters and another son. The average height of the girls in this latter group is 5 feet 6 inches, while the brother is 5 feet 8 inches tall. Both parents are alive, seemingly entirely normal people as are the siblings noted above. The maternal grandfather is reported as having been 6 feet 2 inches tall and the mother, herself of average stature, has three brothers all over 6 feet in height.

4 3 2 7

None of the three dwarf girls has matured but one of them, D., the largest of the group, complains of feeling unwell at monthly intervals and remains in retirement for 2 or 3 days. The brother states positively that there is no flow and that the whole performance is a tribute to convention on the part of the sister. The brother, H., states that he has matured and is sexually potent. Reports from other sources render this statement highly improbable.

They all show an excellent level of mentality, the more so as they have been professionally engaged since childhood,

following a necessarily nomadic existence which has offered but limited educational opportunity. All of the group were normal at birth—the youngest, T., is said to have weighed about 7 pounds—but all failed to grow significantly after the age of 6 or 7. An exception possibly should be made in the case of G. who is reported to have gained nearly an inch during the last year.

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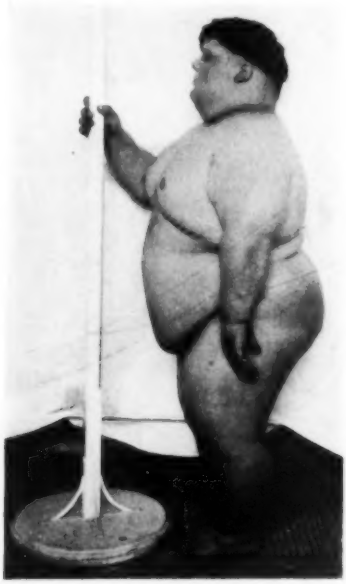
Cases 5 and 6, as previously noted, were not in the original group but were patients seen in years gone by in the diagnostic service of the institution. Both had failed to develop physically and neither had matured. The younger, A.H., has had several later contacts; she has failed to grow significantly and has never matured. Pelvic examination has shown a very marked hypoplasia. These women have been added to the study for several reasons, the first of which being that in size they bridge the gap between the midgets and the normal.

Further, neither of them has matured, thus establishing a parity for comparison in this important respect. Finally, the younger, A.H., is also a case of pituitary arrest, as are the dwarfs in the professional series. The other, M. H., was a premature child and presents a constitutional inferiority in no way associated with primary ductless glandular involvement. There is a definite probability of a congenital specific



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factor which could not be verified as lumbar puncture was denied. Comparison of the respiratory metabolism of this individual with that of the others, all endocrine cases but superficially at least so like her, offers a control for the more precise interpretation of the results obtained.

No. 9, the 'fat lady,' had a family history of obesity, the father, a man of 6 feet, weighing 235 pounds, while the mother, only ½ inch less than her husband, weighs 400. R. is an only

child. She reports herself as weighing 16 pounds at birth and she has continued to gain ever since. She matured at 11, and the menstrual history as reported is entirely normal in all details. She is a graduate both of high school and of a business college.

No. 8, the fat boy, B.G., was first studied at the age of 10 at which time he weighed nearly 400 pounds. Pituitary medi-

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cation coupled with reasonable dietary restraint (hospitalized) reduced him to a level at discharge of 142.3 kilo. Discontinuance of his opotherapy and his diet soon brought him back to his original level and at the time of last contact, here reported, he was just short of 190 kilo. He has since died and the newspaper report of his death gave his weight as 250 kilograms—a value that seems highly probable.

The final member of the original group, no. 10, is the giant, J.E. Beyond his great height, largely achieved between his

eighth and sixteenth years, he presents a relatively normal history. A number of his forebears were very tall people but none of them, so far as he knows, even approximated his own towering altitude. He too is a high school graduate and in his subsequent professional career has availed himself of the educational opportunities afforded by extensive travel.

It is a point to be emphasized that all of this group with the exception of the fat boy, no. 8, were of an excellent intellectual capacity which enabled them to cooperate fully in the performance of the various tests. In the case of B.G., we had a series of extended contacts with him over a period of 4 years and educated him as a subject for metabolism measurements, in which capacity he finally became very proficient.

Turning now to the actual oxygen consumption of the several members of the group, the data are collected in table 2.

TABLE 2

Observed metabolic rate

NO.	NAME	OXYGEN PER MINUTE	CALORIES		
			Per hour	Per square meter per hour	Per 24 hours
1	L.G.	79.0	22.9	35.8	549
2	T.E.	76.6	22.2	29.4	532
3	G.E.	82.8	24.0	29.1	575
4	D.E.	109.4	31.7	31.9	760
5	M.H.	187.4	54.3	42.1	1302
6	A.H.	131.5	38.1	32.9	914
7	H.E.	90.0	26.0	33.4	625
8	B.G.	360.1	104.3	41.9	2520
9	R.P.	356.4	103.2	39.6	2476
10	J.E.	419.7	123.7	38.5	2969

For the first seven members of the series, and for no. 9, the closed circuit method was followed, using the original type of Benedict-Collins' ('20) apparatus with the internal motor, the form which in my experience yields the most satisfactory results. During his periods of study, both the closed and open circuit methods were used with B.G., the results obtained by both methods being mutually concordant.

Due to an earlier unpleasant and unsuccessful experience with a small closed circuit apparatus, J.E. expressed a preference for the open circuit approach and a series of measurements were made with the Tissot spirometers, analyzing the respired gases with the Carpenter modification of the Haldane apparatus. This approach permitted the measurement of the respiratory quotient, in the present instance valuable chiefly as an index of the basality of the patient's state. As noted above, he had had an unfortunate experience some years earlier and the memory of this, operating on a general condition of nervous instability, engendered some disturbance during the first measurement. This gave an oxygen consumption of 449.8 cc. per minute with a respiratory quotient of 0.97. During the second test, he was much more at ease and the oxygen consumption fell to 439.2 with an R.Q. of 0.89. The third essay lowered the oxygen still further to 419.7 cc. but with unchanged quotient. The figure given for the 24-hour heat production in the table above is based upon the calorific equivalent for oxygen at the R.Q. of 0.89 or 4.912 Cal. In my opinion, although the repetition of the test had done much to sedate the patient, he was still slightly nervous and his higher carbon dioxide output was an expression of a respiratory effort still in excess of his needs. Calculating heat production on the basis of the conventional R.Q. of 0.82, the heat exchange becomes 2916. While this is probably nearer his basal requirement, I do not feel that any of the measurements were truly basal and am certain that his real requirement is inferior to the lowest here recorded. It would be inapposite to interpolate any extended discussion of the influence of nervous tension on the apparent oxygen requirement but any one who has had experience with the vicissitudes of clinical calorimetry will recognize the magnitude that such errors may assume. Recognizing, then, that the value reported for J.E. is probably superior to his basal requirement, I have preferred to use the data which the actual measurements warrant.

Taking the first seven members of the series as presenting various stadia of substantially the same physical status, i.e., arrested development, and using the standing height and weight as convenient and significant correlation factors, the energy requirement can be calculated in terms of the units of height and weight.

TABLE 2 a

NO.	NAME	TOTAL CALORIES DIVIDED BY	
		Height (cm.)	Weight (kg.)
1	L.G.	5.78	33.7
2	T.E.	4.96	27.3
7	H.E.	5.80	29.6
3	G.E.	5.06	26.4
4	D.E.	6.04	26.8
6	A.H.	6.45	27.4
5	M.H.	9.30	29.7

While the empirical relationship with height fails to show any marked constancy—a fact which could have been predicted from the probable individual variations in the level of pituitary function—they are all of the same order. This is emphasized by the complete lack of agreement of the coefficient for M.H., the only member of the group with a normally functioning gland.

On the other hand, the comparison on the basis of weight shows a much more definite constancy; here no. 5 falls within the group, while L.G., a clear-cut pituitary case, furnishes the sole significant departure from the mode. As a matter of fact, both of these approaches are artefactual as they, of necessity, fail to consider varying levels of the gland function and other matters pertinent to the influences operating to produce the observed oxygen requirement.

While absolute values are significant as representing the specific performance of the individual, they assume their chief meaning when used as the basis for comparison with those quantities which have been calculated from the prediction formulas of various authorities as indicating the probable

levels of normal performance. In order that these criteria of the normal may be justly representative of the several schools of thought, I have selected those offered by Harris and Benedict ('19), by Aub and DuBois ('17) using the surface area formula of the DuBois and DuBois ('16), the Boothby and Sandiford ('29) modification of the original Aub-DuBois standards, and finally, those offered by Dreyer ('20).

In addition, as the physical characteristics of the dwarfs partake of those weights and areas usually associated with childhood, I have made rough comparisons from the curves for these correlations as prepared by Benedict and Talbot ('21). For purposes of a convenient unity, all standards have been computed to yield normal energy requirement in calories for a period of 24 hours. While this is essentially a convention and does not actually reflect the conditions of living for that period, custom has sanctioned it as acceptable. Further, it can be translated into any other method of expression by simple arithmetical adjustment.

TABLE 3

Normal prediction

NO.	NAME	H.-B.	A.-DUB.		MOD. A.-DUB.[1]		D.
			Unit	Prediction	Unit	Prediction	
1	L.G.	884	37.0	567	36.9	566	769
2	T.E.	956	38.0	687	37.3	674	844
3	G.E.	914	36.5	721	36.2	715	820
4	D.E.	1038	37.0	880	36.6	871	970
5	M.H.	1236	37.0	1145	36.9	1142	1241
6	A.H.	1157	40.0	1112	37.4	1039	1114
7	H.E.	686	39.5	740	39.8	746	905
8	B.G.	3313	46.0	2748	46.2	2760	3017
9	R.P.	2410	37.0	2402	36.9	2396	2330
10	J.E.	3279	39.5	3048	40.3	3110	3051

[1] As changed by Boothby and Sandiford ('29).

As was to be expected, the prediction values show a wide variation. In the main, the Harris-Benedict normals spell one extreme and the two sets based upon surface area the

other. A sole exception to this is in the case of the male dwarf, H.E., whose H.-B. prediction is significantly the lowest of the four. That the two surface area values should show excellent agreement is inevitable, as the modification consists solely in changing slightly the original values for the heat production per square meter per hour. Dreyer's predictions fall between the two extremes—a fact again to be anticipated from the sources of his data and its method of handling. A sole significant exception is the case of the male dwarf, H.E., where the H.-B. is the lowest and the Dreyer significantly the highest prediction value.

As was noted above, rough comparisons have been made where possible with the Benedict-Talbot values for weight and area. The predictions have been taken from the curves and lack the precision of the other calculated values.

TABLE 3 a

Benedict-Talbot predictions

NO.	NAME	WEIGHT	SURFACE
1	L.G.	705	660
2	T.E.	795	755
3	G.E.	850	825
4	D.E.	1005	960
5	M.H.	....	1265
6	A.H.	1125	1125
7	H.E.	900	845

The agreements here are good, the common origin of the two methods of approach rendering this certain.

Turning finally to the comparisons of the rates actually observed with those predicted by the several formulae, we have an approach at least to the function levels of the individuals composing this group.

Dealing first with the dwarfs, there can be no question that they all show a downward tendency to the energy requirement which assumes very concrete proportions in several instances. The smallest of the group, L.G., shows a lack of agreement between the two surface area formulae which are normal,

TABLE 4

Deviations from prediction (per cent)

NO.	NAME	H.-B.	D.	B.-T.		A.-DUB.	A.-DUB. MOD.
				Weight	Surface		
1	L.G.	−38	−29	−22	−17	−3	−3
2	T.E.	−44	−37	−33	−30	−23	−21
3	G.E.	−37	−30	−32	−30	−20	−20
4	D.E.	−27	−22	−23	−21	−14	−13
5	M.H.	+5	+5	....	+3	+14	+14
6	A.H.	−21	−18	−19	−19	−18	−12
7	H.E.	−9	−31	−31	−26	−16	−16
8	B.G.	−24	−17	....	....	−9	−9
9	R.P.	+2	+6	....	....	+3	+3
10	J.E.	−11	−4	....	....	−4	−6

and all of the other predictions which are significantly depressed. The E. family are all consistently low, the Harris-Benedict comparison of the brother being the only one that falls inside the conventional normal boundaries. It is perhaps meaningful that the degree of the depression in the three girls parallels that of the arrest in their development. D., the middle sister, is the tallest and shows the least depression although even she is at a frank hypofunctional level. The interpolated pituitary case, no. 6, A.H., approaches the levels shown by D. The other interpolated case of arrest, M.H., with no evidence of endocrine involvement is above prediction in every instance, and approaches the upper normal boundary in the case of the two surface area predictions. The biometric differences between this patient and A.H. are slight, and again between the latter and the largest of the E. family, G., the gap is not unbridgeable. While this paper was in preparation, a patient was referred to the clinic in the person of a woman of 29, whose height is 135.3 cm. while her weight is 45.0 kilo. Her observed energy requirement was 964 cal., the Harris-Benedict prediction 1200 cal., and the modified Aub-DuBois 1107 cal. These correspond respectively to −20 per cent and −13 per cent below prediction. It has been the practice of this institution to compare observed rates with both the Harris-Benedict and Aub-DuBois[1] standards, taking

[1] More recently the Boothby-Sandiford modification has been substituted.

the mean of the reported value. On this basis the patient shows a rate of — 17 per cent strictly comparable to that of A.H. Thorough diagnostic study of this latter patient shows a long-standing pituitary dysfunction to be the etiological background of her underdevelopment. She has matured, however, and has had one miscarriage together with one child who died 5 minutes after delivery. An acquired venereal factor is undoubtedly responsible for these mishaps. The case is introduced here as she bridges still further the gap between the dwarfs and normal sized adult individuals and offers further warrant for confidence in applying conventional normal standards to this group of pituitary developmental arrests.

Turning to the three remaining cases, we have the two obese individuals, both of whom have a pituitary background. In the case of the boy, there is a long-continued record of study covering intermittently a period of over 4 years. His energy requirement was always below prediction, and in some of the reports during this interval fell to levels very appreciably below the one recorded in this study. The girl, R.P., is in a present transition state and while her true level is certainly inferior to that recorded, she is probably for the moment not far below prediction. The agreement between the several comparisons is interesting.

The final case, the acromegalic giant, gave values which were certainly too high. This point has already been touched upon in the body of the paper. He, too, represents a transition phase of glandular activity in which an initial overproduction of at least the growth hormone is now spontaneously undergoing functional involution to a terminal state of hypofunction.

While all of these measurements are open to the criticism that so warrantably applies to such records with all unconditioned subjects, they yet are maxima and the establishment of a truer physical and mental relaxation would do no more than lower still further readings already low.

In an early paper, Aub and DuBois ('17) had reported the results of studies of a group of individuals of unusual configuration which included five dwarfs. One of these (P.W.) was rachitic, two (R.deP. and S.G.) were achondroplastic, and two others (I.E. and G.F.) were endocrine cases. In addition, they studied the case, J.P., a 17-year-old boy reported by McCrudden and Lusk ('12–'13) as a case of Herter's ('08) 'intestinal infantilism.' The data from one of the achondroplasics (S.G.) do not lend themselves to further scrutiny as the patient was not well and the test abruptly terminated. The data from other five subjects have been calculated in terms of the present approach and the significant findings are collected in the next table.

TABLE 4 a

Analysis of Aub-DuBois data

NAME	AGE	HEIGHT	OBSERVED (INDIRECT)	H.-B.	D.	A.-DUB. (MOD.)
P.W.	38	123.8	1180	941 + 25%	1172 + 1%	1129 + 4%
R.deP.	35	134.7	1256	1066 + 18%	1240 + 1%	1167 + 8%
I.E.	32	134.0	828	1034 — 20%	1200 — 31%	1118 — 26%
G.F.	48	148.9	1159	1216 — 5%	1354 — 14%	1370 — 15%
J.P.	17	113.3	746	811 — 8%	986 — 24%	853 — 13%

The same inconsistencies in prediction values are found here as in the other series. At the same time, the general trend of the indications is unmistakable. P.W. and R.deP., cases without ductless glandular involvement, show values above prediction and with the exception of the H.-B. values, well within normal limits. I.E. was an endocrine case (hypofunctional) and shows a characteristic depression of the respiratory metabolism. G.F., also an endocrine case, gives less well-marked evidences of departure from the normal, so

far at least as the respiratory exchange is concerned. He is, however, 48 years old, and his height non-significantly below 5 feet—in other words, the energy requirement is consistent with his pituitary status. The last case, J.P., without glandular involvement, but in a state of marked malnutrition, shows the depression of oxygen use which reflects the nutritive status. This group of cases, studied independently and by both direct and indirect calorimetry, yield values which are wholly consistent, in relation to the physical status, with those of the present series.

As was noted in the beginning, certain other aspects of the studies on this interesting group will be reported elsewhere. The present paper is confined to the report of the respiratory metabolism.

One other group of observations needs be recorded. In all clinical metabolism measurements there are a number of significant physical records that constitute an essential complement to the measurement of the oxygen consumption. From these records, taken at intervals throughout the time of contact with the subject, some estimate can be made of the degree of relaxation at the time of testing, and hence of the authority of the energy consumption report.

TABLE 5

Complementary physical data

NO.	NAME	BLOOD PRESSURE		PULSE	RESPIRATION	TEMPERATURE
		Systolic	Diastolic			
1	L.G.	94	68	80	10	98.0
2	T.E.	88	52	76	12	98.4
3	G.E.	106	80	64	14	98.0
4	D.E.	86	50	64	15	98.0
5	M.H.	102	58	76	16	99.2
6	A.H.	102	70	84	14	98.0
7	H.E.	98	72	79	16	98.6
8	B.G.	164[1]	90[1]	64	16	97.2
9	R.P.	160	90	62	7	97.8
10	J.E.	146	90	78	10	98.6

[1] Known cardiorenal condition.

In the main, these values warrant confidence in the relative accuracy of the energy metabolism reports. Case 5, M.H., has a slightly high temperature but it will be remembered that her comparisons were all slightly above prediction. Correction to a conventional 98° will still leave them all within the normal zone. The fat boy was a cardiorenal case and seemingly the fat girl has an early hypertension. The somewhat high diastolic pressure of the giant is in harmony with the hypertensive influence of pituitary overactivity. Case no. 6, A.H., shows a pulse somewhat above her usual level. Broadly speaking, the pituitary dwarfs show hypotension and are otherwise fairly normal. This is in complete accord with the usual clinical experience.

To conclude, we have here a record of the respiratory metabolism of a group of individuals all of whom depart significantly from conventional physical standards. The basal rates, in general, reflect the physical status that has engendered their abnormality. Further studies, which it is hoped to make, will probably show somewhat lower values, but it is questionable if the further depression will assume magnitudes significantly different in comparison with those here recorded.

I wish to express my deep appreciation of the courtesy and friendly cooperation on the part of the subjects which made these studies possible, recognizing that it entailed a sacrifice of leisure and of personal convenience.

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THE TOXAEMIAS OF PREGNANCY

III. THE RESPIRATORY METABOLISM

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TWO FIGURES

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In a series of earlier communications (Rowe, '32; Rowe et al., '33), the senior author and his associates have discussed certain results derived from the study of fifty-two pregnant women exhibiting some one of the toxaemias which may complicate the physiological condition.

The present report is concerned with the respiratory metabolism together with certain other observations germane to its discussion. The general method of approach, composition of the group, and similar material, have already been presented in the earlier papers and need not be detailed here. It may be noted, however, that each of the group was studied at some time ante-partum and again within 2 weeks after terminating the pregnancy. All of the women were toxic, one died 9 days after delivery, and two more died during the initial study, the records being so incomplete that they have been deleted from the series. As the relationship of the time of initial study to that of delivery is pertinent to certain aspects of the discussion, the data are briefly presented.

TABLE 1

Group	*Number*
A. Delivered at time of study,	33
B. Delivered within 1 month later,	6
C. Delivered from 6 weeks to 6 months later. Average 3.5 months,	11
	50

Two-thirds of the series were delivered spontaneously or by induction at the time of first hospital entry; six more terminated the pregnancy within a month of the initial study. With the remainder, one-fifth of the group, treatment permitted a continuance of gestation. Of the eleven children in this group, two were delivered at term spontaneously, one of which was a stillbirth, while the other survived only 16 hours. Labor was induced in two others, delivering a dead foetus at 6.5 months in one, and an 8 months' child which survived 6 days in the other. The remaining seven children survived. The complete record of the outcome of pregnancy is given in the next table.

TABLE 2

Outcome of pregnancy. Toxaemia series

DATUM	DELIVERY			
	Spontaneous	Induced	Total	
Premature, survived	4	5	9	25
Premature, dead	2	7	9	
Premature, died later	2	5	7	
Full term, survived	13	8	21	25
Full term, dead	2	1	3	
Full term, died later	1	0	1	
	24	26		

Twelve pregnancies yielded dead children ranging from a therapeutic abortion of a 2½ months foetus to the child stillborn at term. Eight more were living at the time of delivery but died after intervals ranging from 5 hours to 28 days. A more complete analysis of these two groups will be given in the body of the paper in connection with the respiratory exchange.

Prior to undertaking the investigation of toxic pregnancy, a series of standardizing studies on normal pregnant women was conducted by the senior author and his associates. The results have been summarized (Rowe, '31) and later reference will be made to these studies of the normal as they serve as the control material for the present investigation. During

pregnancy, a variety of mechanisms are engendered which are associated with the underlying physiological condition and which terminate with delivery. The growth of the foetal tissues, the storage of protein by the maternal organism in excess of foetal needs, and a variety of other processes all influence the energic demand of the maternal organism which during the period of gestation comprehends them all. That the added foetal metabolism alone is not competent to account for all the changes recorded in the measurement of the energy requirement of the mother, has recently been shown by one of us (Rowe and Boyd, '32). The growing foetus, however, does make a significant and highly important contribution to the factors which can be measured only in summation. Weights at delivery, as potential rough measures of the foetal contribution, assume a very real importance for present considerations. Average values for the several groups have been collected; the average weight of the seventy-seven children in the normal series is included.

TABLE 3

Weight relations, child

DATUM	NUMBER	WEIGHT (AVERAGE)
		kg.
Premature child, dying after delivery	7	2.15
Same, surviving	9	2.62
Full term child, surviving	21	3.38
Same, normal series	77	3.27

The stillborn children, twelve in number, have been omitted as the records are incomplete and the scatter of weight and period of gestation is scarcely significant in that portion where report was made. The record is omitted from the table also of one child spontaneously delivered at term which survived 16 hours. This was the largest baby in the series, weighing 4.34 kg.

The non-surviving premature children present one definite handicap in an average weight nearly one-fifth less than that

of the surviving series. The full term children, on the other hand, show a slightly greater average weight than the babies of the normal series, and this in spite of the subversive influence that the mother's toxic state might be presumed to exercise on foetal growth. While the exact degree of prematurity must be a matter of estimation and so lacks any real precision, the weights as recorded are equal or slightly superior to those reported by Scammon and Kline ('30). A quantitative comparison is impossible but qualitatively at least the surviving children have shown a normal development in spite of the mother's condition; one explanation of the lighter non-surviving group could patently lie in a greater prematurity than their surviving fellows.

While the foetal weight cannot be recorded with precision, that of the mother is not subject to this disability if certain reasonable precautions are taken to render the records of a series comparable with each other. Further, the maternal weight summates all of the tissue changes and accretions deriving from the fact of the pregnancy. The data from all of those with complete records are collected in table 4. The complementary values from the normal group are added for purposes of comparison.

TABLE 4

Maternal weight relations

DATUM	NUMBER[1]	WEIGHT (KILOGRAMS)		LOSS
		Ante-partum	Post-partum	
Delivered:				
At term	9	78.9	66.6	12.3 (16%)
1 month premature	8	72.0	62.2	9.8 (14%)
2 months premature	12	68.4	59.6	8.8 (13%)
Over 2 months premature	10	59.5	58.9	0.6 (1%)
Normal:				
At term	77	66.3	57.4	8.9 (13%)
1 month premature[2]	77	64.4	57.4	7.0 (11%)
2 months premature[2]	77	62.5	57.4	5.1 (8%)

[1] In eleven of the toxic patients, only single weights could be secured, which unfits them for this comparison.

[2] Calculated from observed weekly coefficient of 0.48 kg.

In the toxic group, one finds those increments of weight with the progress of gestation which are to be anticipated. They are somewhat greater in the toxic than in the normal group as gauged by the average weights 2 weeks post-partum. The warrant for computation by a linear formula for the latter group has already been established in the paper previously cited (Rowe and Boyd, '32). While absolute values offer basic data, relative changes at times disclose an underlying trend with a greater clarity.

Some years ago, Dreyer ('21) computed a series of relationships from observations on a group of normal individuals which are of real service, within certain limits, in the prediction of certain normals. Dreyer calculates weight from sitting height ('trunk length') and chest girth, the former uniformly valid with reasonably normal sitting height indices, the latter applicable only, as one of us previously has pointed out (Rowe, '25), to people of relatively normal configuration. The normal sitting height index (sitting height divided by total height) shows an average of 0.533 for adult women in this part of the world. Reasonable deviation within the normal could lie between 0.52 and 0.54, and with these indices Dreyer's predictions are to be regarded as reliable, as are the measurements from which they derive. In the present group of toxic mothers, the indices are grouped as in the next table.

TABLE 5

Sitting height indices

Index	0.50	0.51	0.52	0.53	0.54	0.55
Number	3	5	12	19	9	2

In other words, 80 per cent of the group falls within the rather limited arbitrary normal boundaries and the remainder, while their indications are somewhat erroneous, cannot influence the averages of the entire series to a disturbing degree. Translating the absolute values of table 4 into the relative magnitudes based upon Dreyer's sitting height ('trunk') predictions, the results appear in table 6.

TABLE 6

Deviation of maternal weight from predicted normal (Dreyer)

DATUM	NUMBER	WEIGHT DEVIATION (PER CENT)		
		Ante-partum	Post-partum	Loss
				Per cent
Delivered:				
At term	9	+ 38	+ 16	22
1 month premature	8	+ 34	+ 16	18
2 months premature	12	+ 35	+ 17	18
Normal series	77	+ 15	+ 1	14

The first and most significant fact that this approach discloses is that the members of the toxic group are definitely overweight. Not only are the departures ante-partum of an order that indicates a real obesity, but the deviations after delivery, in spite of the high weight losses as given in table 4, indicate a group of individuals who are still much above the normal. The average post-partum excess is actually greater than the recorded deviation of the normal group just before delivery. Further, it contrasts strikingly with the practical coincidence of observed and predicted values in the normal series after the pregnancy has terminated. The toxic cases were obese to begin with, they added a disproportionate amount of weight—only attributable in part to foetal growth —during gestation and they remain significantly overweight after delivery. The post-partum comparisons are susceptible to a further detailed analysis and these data are collected in the next table (table 7).

TABLE 7

Relative maternal obesity post-partum

DATUM	NUMBER	AMOUNT (AVERAGE)
		per cent
Below prediction	12	— 10
From prediction to + 15 per cent	12	+ 10
Over 15 per cent above prediction	18	+ 32

Only twelve of the forty-two patients with complete post-partum records are below prediction, and that only with the

modest average of — 10 per cent. On the other hand, 43 per cent are not only above the liberal allowance of + 15 per cent, but their average deviation is + 32 per cent—a very tangible degree of overweight.

Now it is to be remembered that all of these women were toxic, giving a definite number of evidences of markedly disturbed function levels. Gastrointestinal disturbances were common—an agency that should lower rather than augment weight. Naturally, edema was a common finding, and this might be conceived to account for a part at least of the overweight, and, moreover, a part that could prove to be a disturbing source of error in the proper prediction of the energy metabolism. But the overweight persists after the uterus has been emptied and the edematous accumulation in largest part cleared up. Naturally, inert water trapped in the tissues will influence subversively the accuracy of any prediction in which weight plays a part. It is, however, only one factor and thus does not appear in the prediction value as more than a fraction of its absolute amount. To illustrate, 20 kg. of water from edema, raising the weight from 58 to 78 kg., would change the weight component of the energy prediction by the Harris-Benedict ('19) formula from 1210 to 1401—an error lessened when the age-height increment is added to make the total. For this group it would be of the order of 14 per cent.

A similar approach, using the DuBois ('17) surface formula, would increase the area of a 160 cm. woman (the average for this series) from 1.60 to 1.81 square meters, or about 13 per cent. Edema, then, while a factor, cannot enter into the picture in a magnitude which assumes disturbing proportions. In the post-partum period, it seems reasonable to say that its potential effect is wholly negligible.

In addition to his weight predictions, Dreyer (l.c.) has also calculated standards of normal lung volume. Here again his limitation to individuals of relatively normal configuration makes his standards based upon weight and chest circumference worthless for those who do not comply with this

criterion. The correlation with sitting height, within the limitations already noted, gives values of greater applicability.

In the paper previously noted (Rowe, '31), one of us has described the increase in lung volume exhibited by the normal woman as her pregnancy progresses. Taking a representative linear translation of this curve as a standard, we have

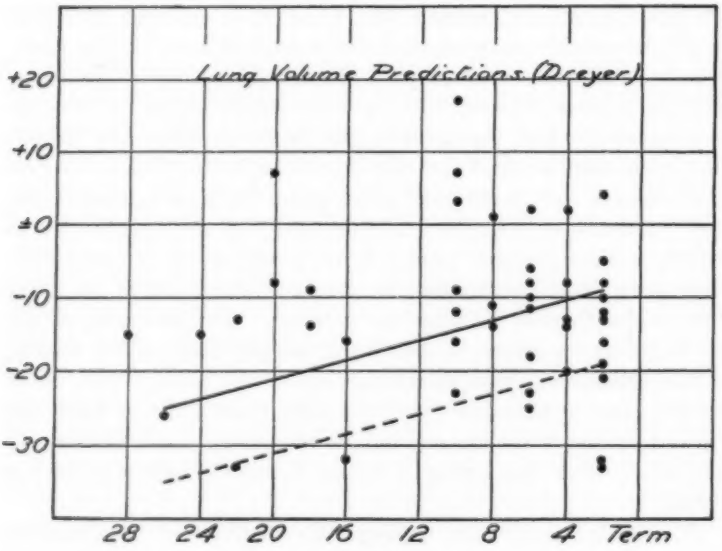

Chart 1

made a scatter diagram of the reports of lung volume deviation derived from this present toxic series (chart 1).

The heavy black line is the characteristic taken from the data of the normal series; the dotted line, a boundary 10 per cent below this standard which may be conceded to indicate a reasonable lower limit for normal performance. It is evident that but a small number (Rowe, '31) of the entire series fall below this lower boundary, and further, that twenty-five of the forty-five points are superior to standard curve. In other

words, there is no reliable evidence here to indicate a lessening of lung volume as a result of the toxic state, and during the earlier months of gestation, at least, the values for the toxic are superior to the normal average. Even at 9 months, in this the most heavily downward weighted series of observations, the normal average is —9 per cent, and that of the toxic group —15 per cent. The average deviation of the toxic group, usually about 12 days post-partum, is the nearly normal figure of —12 per cent.

During the standardizing studies on the normal group, basal rates were measured at intervals of 2 weeks from the time of initial contact to some 3 months after delivery. The composite of these measurements has defined an average curve of the deviations from prediction throughout gestation. During the last 24 weeks before delivery, these averages define a linear upward trend from a point 8 per cent below to one 5 per cent above prediction. Taking this median as the standard and defining conventional boundaries of normal deviation at 10 per cent above and below, it has been possible to construct a scatter diagram of the basal rates actually recorded with the toxic series (chart 2).

A few words of explanation may be apposite. It has been the practice of this institution in reporting basal rate measurements to compute the normal prediction by both the Harris-Benedict ('19) and Aub-DuBois ('17) equations and record the mean deviation of the two comparisons as that of report for the test. More recently, we have substituted the Boothby-Sandiford ('29) values for heat production per square meter per hour for the earlier Aub-DuBois standards. In the present instance, however, the earlier practice has been followed throughout.

To the informed group under whose scrutiny this paper will fall, it is superfluous to discuss at any length the sources of error in the measurement of the so-called basal rate with individuals who have not been conditioned to the test. Further, the patients in this group, as has already been emphasized, were sick, many of them gravely so, and this

offered a still further hindrance to obtaining authoritative results. Recognizing the subversive factors intrinsic in work of this character, every effort has been made to minimize the sources of error as far as possible. The patients were all hospitalized—a fact of great assistance in the accomplishment of this end; among other advantages, it permitted the

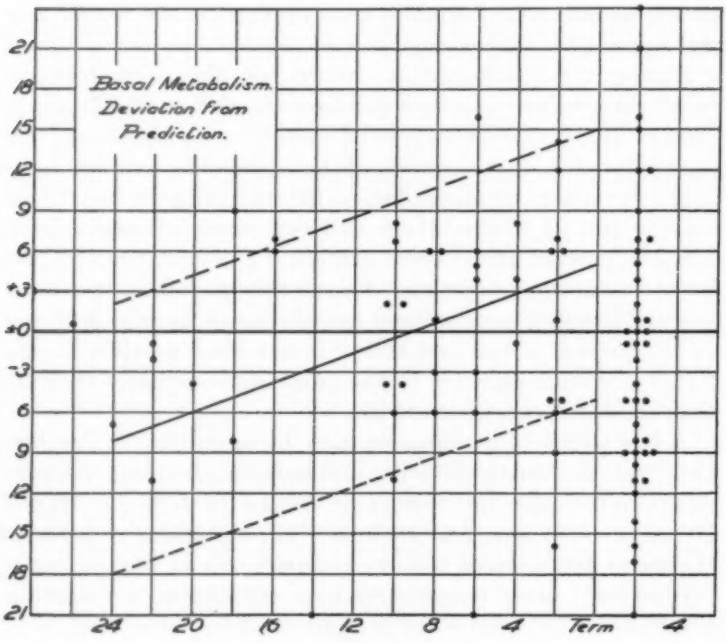

Chart 2

performance of preliminary tests which served, through education, to lessen the psychic factor of disturbance. In one or two instances, the patient was too ill to permit the performance of the test. Early in the series, we did carry out tests on a very sick and highly neurotic woman of 38, recognizing that the results were worthless as an indication of the basal energy requirement. Her best test ante-partum

was + 76 per cent and the level 12 days after the spontaneous delivery of a viable child some few weeks before term was + 27 per cent. She was in a much better state at the time of the second (post-partum) test but was obviously in a far from basal condition, restless and mentally much disturbed. She did not have a toxic thyroid. With these and the many similar observations deriving from reliable sources, the occasional reports in clinical literature of isolated measurements on obviously far from basal patients offer but meager warrant for the sweeping generalizations so freely drawn from them.

Reverting to our own data as charted in the graph, only nine of the forty-six records fall outside the conventional normal zone, four above and five below. Further, one of the low values and one of the high are but 1 per cent outside of the limiting boundaries and the single value at 28 weeks is probably well within the normal range as the curve of the normal makes a sharp upward excursion at about this point (Rowe and Boyd, '32). In other words, these data indicate that outside of the exceptional case in which some superimposed factor of influence exists, there is no reason to believe that toxaemia in pregnancy produces any characteristic influence on the respiratory metabolism. Two or three of the patients may—and probably did—have a hypofunctional endocrinopathy which would exercise its own influence quite independently of the toxic status.

The values post-partum call for a different standard of comparison. There is a definite although transitory drop in the basal rate which in the normal series gave averages of — 10 per cent and — 15 per cent for two individual subgroups at intervals between 2 and 3 weeks post-partum. In the toxic group, the average interval was 2 weeks or a little less, so that the value of — 10 per cent would be a fair average of normal performance. If this be assumed as the mean value, twenty-six of the forty-one records obtained fall within the conventional normal zone of ± 10 per cent. All of the others, fifteen in number, are superior to prediction and in

a number of cases—chiefly those showing the higher values above prediction—were recognized as not basal and unsatisfactory at the time of performing the test. In other words, again, neither ante- or post-partum do these results warrant any interpretation of an influence of toxaemia on the basal metabolic rate.

While the fate of the foetus is not a rigorous standard for the estimation of the severity of the toxaemia, it seems a reasonable inference that in the cases presenting a dead foetus or one not surviving after birth, the toxicity was severe. Analysis of this special group offers some measure of control over the inferences drawn from the survey of the whole group. These data are collected in the next table.

TABLE 8

Non-surviving babies

NO.	MONTH SEEN	DELIVERY		DEAD SURVIVED	MATERNAL BASAL RATE	
		Type	Time		Ante-partum	Post-partum
3	3.5	Spon.	4	D	−7	−11
5	8	Ind.	8	18 days	+6	+16
10	5.5	Ind.	6	D	+7	—
13	7.5	Spon.	8	12 days	−6	+21
16	8	Ind.	8	28 days	−6	—
21	9	Spon.	9	D	+1	—
22	9	Spon.	9	D	+7	−9
29	4	Ind.	6.5	D	−2	−9
30	6.5	Ind.	7	5 hours	—	−7
31	5	Spon.	9	16 hours	−8	—
35	7	Ind.	7	D	−4	−8
36	7	Spon.	7	D	−4	+12
38	8	Ind.	8	D	−3[1]	—
39	4.5	Spon.	8	6 days	−4	−5
40	8.5	Ind.	8.5	12 hours	−1	
41	2.5	D. and C.	2.5	D	+3	−4
45	7	Ind.	9	D	+8	−10
46	8	Ind.	8.5	2 days	+4	+1
47	7	Ind.	7.5	D	−11	−9
50	7	Ind.	7	D	+7	−21

[1] Mother died after delivery.

The figures scarcely without exception fall within normal ranges. Nos. 5, 13 and 36 are somewhat high post-partum,

but high values lack the definitive meaning of those significantly below prediction. One, no. 50, is possibly low and is one of the cases showing the same level ante-partum. An endocrine factor here is not improbably operative. One striking case is that of no. 38, a primipara of 30 years of age. Labor was induced during the eighth month, the child was stillborn, and the mother died 9 days later. A few days before her delivery, her basal rate was the rigorously normal value of — 3 per cent. The further analysis of these selected cases certainly supports the inference drawn from the survey of the entire group.

Alveolar carbon dioxide is another element of the respiratory exchange significantly affected by pregnancy. During gestation there is a depression of CO_2 tension to levels suggesting acidosis and these continue throughout the period of gestation. The average value for the normal series was 31 mm. with recovery to 35 mm. 2 weeks after confinement. In the toxic series, the average ante-partum was 35 mm., and 2 weeks after delivery 39 mm. The relationships are the same but the toxic cases show more nearly normal values than do the normal. The probable explanation of this abnormality is not immediately forthcoming, in part at least because the initial underlying mechanism has not been satisfactorily explained. The Macallum theory of ketogenesis offers a possible lead, but discussion here is not germane to the main thesis.

There remains one other group of facts which are an essential part of every properly conducted measurement of the basal metabolic rate since they may give valuable indirect information as to the authority of the main observation. These are such physical indices as the blood pressure, pulse and respiration rates and body temperature. Before discussing these results in the present study, one further consideration must receive cognizance. The term toxaemia of pregnancy is no more than a convenient collective caption embracing a somewhat wide variety of abnormalities with only the associated physiological condition as the single, common

factor. In the majority of cases, however, evidences are not lacking of a potent hepatic or renal element, one or both, and as each of these mediates results in some measure independent of the other, due allowance must be made for each in any form of collective presentation. In the previous papers, the cases of this series have been divided into four sub-groups on the basis of hepatic and renal elements in the etiologic picture. Table 9, which follows, defines these for the subsequent discussion.

TABLE 9
Composition of toxaemia group

GROUP	I	II	III	IV
Liver	+	+	?	0
Kidney	0	+	0	+
Number	12	12	12	14
Per cent	24	24	24	28

The uniformity of distribution is fortuitous. In subgroup III, an hepatic element was possible where not actually probable, but the crucial objective evidences to resolve the matter could not be secured. Using these subgroups for greater clarity in presentation, the evidences supplementary to the basal rate record are collected in the next table.

TABLE 10
Supplementary physical measurements

DATUM	I	II	III	IV	NORMAL
Blood pressure:					
Systolic, ante-partum	146	169	148	152	108
Systolic, post-partum	141	140	149	135	112
Diastolic, ante partum	85	105	94	100	67
Diastolic, post-partum	84	90	94	83	70
Pulse, ante-partum	73	78	78	77	78
Pulse, post-partum	78	79	74	75	67
Respiration, ante-partum	14	16	16	16	17
Respiration, post-partum	15	15	15	17	17
Temperature, ante-partum	98°	98°	98°	98°	98°
Temperature, post-partum	98°	98°	98°	98°	97°

All of the groups show a hypertensive trend which, in group II, with one influence supplementing the other, assumes the highest level. Recovery to normal levels after delivery is not a feature of any of them, but the two sub-series with the renal factor show a greater tendency toward conventional normality than do the others with uncomplicated and established or putative hepatic complication.

Pulse rates, initially recording the somewhat more rapid rhythm characteristic of normal pregnancy, fail to recover in the toxic as do those of the normal series after delivery. These augmented rhythms may derive in part from the psychic influences that indubitably are responsible for some of the questionable basal rate measurements on which comment has already been passed. The respiration rates show divergences of so small a magnitude as to be non-significant. There is an upward tendency to body temperature in the toxic patients which seemingly tends to persist after the delivery of the child.

To summarize, these supplementary evidences, largely of a cardio-respiratory origin, give much more clear-cut evidences of the effect of the toxaemias than have the other observations discussed in the body of the paper.

As running comment has been passed upon each set of evidences as they were presented, further discussion is superfluous. The contents of the paper may be briefly summarized as follows:

SUMMARY

Somewhat elaborate study of a group of fifty pregnant women with some form of the toxaemias of pregnancy, observed both before and after delivery, has yielded the following tentative conclusions:

1. The toxic cases exhibit an obesity that persists after delivery.

2. The weight of the children born at term does not depart significantly from those resulting from normal pregnancy. This same generalization seemingly holds true for the pre-

mature births as compared with normal values computed from reliable sources.

3. The lung volumes of the toxic do not depart significantly from those defined as normal for the pregnant state.

4. The respiratory metabolism of the toxic patient is, generally speaking, well within the range of that determined for the course of normal pregnancy. Departures from this are few in number and are to be traced to superimposed effects not demonstrably associated with the toxic status.

5. Alveolar carbon dioxide tensions, while depressed, do not reach the low levels recorded in normal pregnancy.

6. Blood pressures, pulse rates and body temperatures all show concrete changes from the normal traceable to the toxic condition. Recovery to normal levels after delivery is delayed.

The authors express their appreciative thanks to all who have participated in these studies.

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THE EFFECT OF MINERAL OIL ADMINISTRATION UPON THE NUTRITIONAL ECONOMY OF FAT-SOLUBLE VITAMINS

II. STUDIES WITH THE VITAMIN A FACTOR OF YELLOW CORN[1]

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TWO FIGURES

(Received for publication September 1, 1933)

Mineral oil ingestion by an animal under certain experimental conditions impairs the assimilation of vitamin A. In a previous communication (Jackson, '31) we reported extensive experiments demonstrating that the administration of the minimum requirement of butter fat in a mineral oil solution results in a considerable restriction of growth with other attendant symptoms of vitamin A deficiency. These studies confirm those of Dutcher, Ely and Honeywell ('27). It was shown further, however, that if the butter fat and mineral oil were given at different times, the animals gave evidence of only a slight deficiency of vitamin A. Finally, the separate administration of mineral oil apparently produced no untoward effect when the butter-fat dosage was increased by 50 per cent.

While the writer's communication was in press, there appeared a paper by Rowntree ('31) dealing with the same subject. This investigator observed that mineral oil administered with cod liver oil did not interfere with vitamin A absorption. Although no assay values are presented, the

[1] The expenses of this investigation were defrayed from the income of the Mineral Oil Fellowship, Yale University, 1929–1930.

quantity of cod liver oil employed appears to be several times the minimum requirement. When the vitamin A intake was limited to something less than this requirement, the incorporation of mineral oil with the vitamin (from 'food extracts') caused the animals to lose weight very rapidly. In general, Rowntree's results appear to be in accord with those of other investigators.

In view of the importance of the mineral oil-vitamin A question relative to the large consumption of mineral oil for the relief of constipation, we have made further studies bearing on this problem. The following report deals with the effect of mineral oil administration on the availability of vitamin A or its precursors supplied in yellow corn.

EXPERIMENTAL PART

Inasmuch as a complete description of and the arguments for the general technic employed in this study have already been presented (reference cited), only the more important items are recounted here.

Animals, diet and depletion method. Male albino rats, ranging in weight from 39 to 46 gm., were weaned at the age of 21 days and given the following vitamin A-deficient diet ad lib.

Composition of the basal diet (without added vitamin A)

	Total weight per cent	*Total calories* per cent
Casein, extracted	18	16
Starch, cooked	63	55
Salts, Osborne and Mendel ('19)	4	..
Hydrogenated vegetable oil[1]	15	29
	100	100

[1] Crisco.

Vitamins B, G and D and other possible growth factors were supplied daily apart from the rest of the ration in the form of 400 mg. of dried yeast (Northwestern Yeast Company) irradiated according to Hess ('27).

Vitamin depletion, as measured by eye condition and more particularly by body weight change, occurred in the average time of 26 days. Whenever it was determined that an animal

was definitely depleted, he was given the full amount of vitamin employed in the experiments (1450 or 2175 mg. of yellow corn) daily for 4 days to prevent the rapid onset of irreparable tissue damage. The definite but temporary effect of this treatment is clearly evident in the growth graphs of the negative control animals (chart 1).

Conduct of experiments. To eight groups of animals, following the period of readjustment described above, were administered respectively the following daily (note below the Sunday exception in the case of the mineral oil): no corn; 725 mg. of corn; 1450 mg. of corn; 1450 mg. of corn and 0.5 cc. of mineral oil no. 1; 1450 mg. of corn and 0.5 cc. of mineral oil no. 2; 2175 mg. of corn; 2175 mg. of corn and 0.5 cc. of mineral oil no. 1; 2175 mg. of corn and 0.5 cc. of mineral oil no. 2. These regimes were continued, if the animals survived, for 75 days. The animals were weighed and the food consumption measured twice a week. Body weight, eye condition and necropsy findings were the principal criteria of vitamin A economy.

The preparation, dosage and administration of the corn and mineral oil. The corn employed was the Improved Leaming Yellow Dent variety of seed quality. The same lot was used throughout the investigation. Although one method of preparing corn for assay is to weigh and sort the individual grains, we found it a better procedure for our purpose to employ the corn in the form of a homogeneous meal from which any desired quantity could be measured. A fresh supply was ground every 2 weeks and kept in the dark at a temperature of about 5°C. Although Marcus ('31) has shown that vitamin A concentrates undergo marked deterioration when dispersed on finely divided particles of various substances, Kick and Bethke ('29) found little loss in the vitamin A content of ground, whole or cracked yellow corn when stored for as long as 1 year. Preliminary assay of the corn was performed (compare groups I, II, III and VI in chart 2), and 1450 mg. chosen as the minimal daily amount which would permit a depleted animal to grow at approximately a normal

rate with disappearance of symptoms of vitamin A deficiency. The vitamin A of this corn apparently falls within the potency range reported in the literature (compare, e.g., Steenbock and Coward, '27, and Meyer and Hetler, '29). Comparison of groups III and VI of chart 2 indicates that 1450 mg. of corn is perhaps slightly less than the amount necessary for maximal growth under our experimental conditions. The corn was measured with an accuracy of $\pm$ 3 per cent.

Two commonly used standard brands of mineral oil, called mineral oils no. 1 and no. 2, were employed. Five-tenths cubic centimeter of oil comparable to a therapeutic dose for man was the daily amount administered to the rats. A short description of the oils and the reasons for the selection of

EXPLANATION OF CHARTS 1 AND 2

The growth curves depicted are of two kinds: chart 1, representative curves plotted in detail to serve as protocols, and chart 2, straight-line graphs to facilitate group comparison. The latter were prepared by drawing lines from an arbitrary common origin representing 40 gm. to points representing the final weights (in grams) attained. The corresponding abscissae represent the average depletion time, 26 days, plus the recuperation interval, 4 days (see text), plus the experimental period, 75 days; a total of 105 days. The black columns show averages of group food consumptions in grams for the experimental period. Of the two arrows shown in the protocol curves, the first records the time when the animal was adjudged to be definitely depleted of vitamin A; the second, the end of the 4-day readjustment period.

The Roman numerals indicate groups of animals receiving yellow corn and mineral oil in daily dosages as follows:

I. No corn.
II. 725 mg. of corn.
III. 1450 mg. of corn.
IV. 1450 mg. of corn and 0.5 cc. of mineral oil no. 1.
V. 1450 mg. of corn and 0.5 cc. of mineral oil no. 2.
VI. 2175 mg. of corn.
VII. 2175 mg. of corn and 0.5 cc. of mineral oil no. 1.
VIII. 2175 mg. of corn and 0.5 cc. of mineral oil no. 2.

The cross designates death before the termination of the experimental period of 75 days. Other symbols adjacent to the ends of the curves indicate necropsy findings:

E. Definite ophthalmia.
T. Abscess formation in the tongue.
S. Abscess formation in the submaxillary glands.
K. Calculi in the kidneys.
B. Calculi in the urinary bladder.

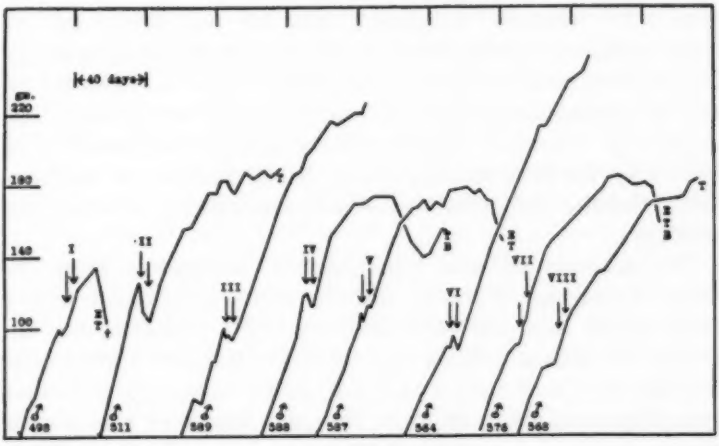

Chart 1

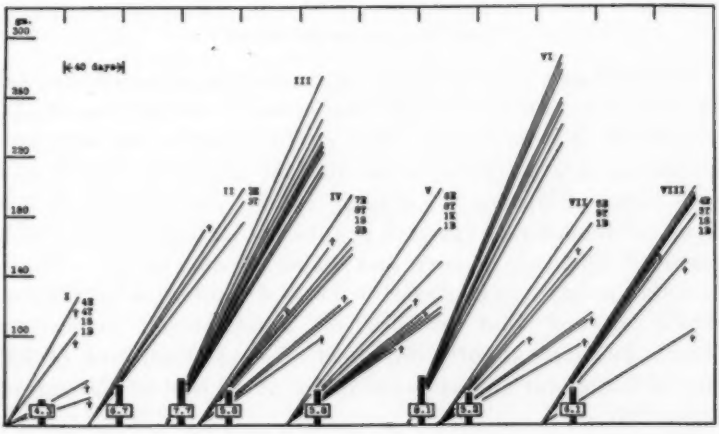

Chart 2

this dosage have been given previously and need not be rehearsed here. It is of interest, however, to note that Rowntree ('31) likewise estimated that 0.5 cc. of mineral oil 'approximates two tablespoons for the human being' and employed experimental doses from 0.25 to 0.75 cc. The 0.5 cc. of oil administered daily to our rats did not produce any degree of diarrhea. However, the oil was responsible, as shown by the time necessary for the elimination of markers, for a definite but rather moderate acceleration of intestinal motility.

The mineral oil was administered—separately from the corn—by means of a 1-cc. insulin syringe equipped with a clean glass tube for each delivery. The schedule for the giving of mineral oil was as follows: 0.17 cc. daily except Sunday at 8 to 9 A.M., 2 to 3 P.M. and 5 to 6 P.M. The corn was dispensed daily at 11 to 12 A.M. The corn was not ingested avidly as was the butter fat, but ordinarily disappeared during a period of 3 hours and was not left over to the following day.

DISCUSSION OF RESULTS

Charts 1 and 2 show clearly that administration of mineral oil seriously interferes with the animals' utilization of the vitamin A of yellow corn. This is true whether the corn was employed in a marginal intake of 1450 mg. or in one 50 per cent larger, 2175 mg. A similar finding was secured with a series of female rats (growth graphs not included here) which received 1450 mg. of corn and 0.5 cc. of mineral oil. These results are quite in contrast to those of the butter-fat studies which showed that mineral oil administered separately diminished only slightly the effect of a marginal dose of 100 mg. of butter fat and apparently not at all that of 150 mg. of butter fat.

Inasmuch as the corn was not eaten immediately, as was the butter fat, a series of male animals was depleted of vitamin A and then given the 0.5 cc. of mineral oil in three doses at 8 to 9 A.M., 11 to 12 A.M. and 2 to 3 P.M. and the 2175 mg. of

yellow corn at 5 to 6 P.M. Thus the last dose of mineral oil was administered 3 hours before the corn with a 14-hour period following before the next dose of mineral oil. These measures, instituted to insure a better separation of the corn and mineral oil in the gut, did not alter the outcome of the tests. The growth graphs are very similar to those depicted in groups VII and VIII in charts 1 and 2.

One suggestion growing out of these experiments was that the vitamin A potencies of the corn and the butter fat were due to two different substances with different properties. Recently, Dutcher, Harris and Guerrant ('33) have projected such a conception based on the discoveries of the last few years concerning the natures and physiological relationships of carotene and vitamin A and explain somewhat similar results of theirs as follows:

> In a previous publication from this laboratory, it was shown that rats fail to show typical vitamin A response when butter fat is fed in the presence of mineral oil. Vitamin A in cod liver oil, however, is used quite satisfactorily under similar experimental conditions. The present experiments offer a possible explanation for the results just described. Carotene (dissolved) in corn oil is not utilized in the presence of mineral oil, while a carefully prepared pigment-free vitamin A concentrate (from cod liver oil) is utilized readily when fed with mineral oil. The hypothesis is advanced that these results are due to preferential solubility of hydrocarbon (carotene) in hydrocarbon (mineral oil) and the preferential solubility of sterol (vitamin A) in the sterol containing secretions of the intestinal tract.

However, Baumann and Steenbock ('33) have recently reported that: "In the sum total of biological activity (of butter) carotene is not of major importance, since it accounted for only 15 per cent of the total." It appears, therefore, that the hypothesis of Dutcher, Harris and Guerrant could apply to only 15 per cent of the butter-fat potency, i.e., the carotene portion. These writers do not state whether their pigment-free vitamin A concentrate and carotene were fed under the comparable condition of approximately equivalent dosage.

Different writers have made the reasonable observation that the amount of vitamin A fed is an important factor in determining the effect of the mineral oil. In other words, a sufficient excess of the vitamin seems to surmount the effect of the mineral oil. At all events, their hypothesis itself appears to be supported by our experiments wherein the mineral oil interfered with the utilization of the vitamin A factor to a much greater degree in the case of the corn than in that of the butter fat. Butter fat, according to Baumann and Steenbock, is 85 per cent vitamin A,[2] while the vitamin potency of the yellow corn like that of other plant products is presumably due to the presence of carotene.

The experiments herewith described showing that the separate administration of mineral oil distinctly inhibits the assimilation of the vitamin A factor in yellow corn do not favor the indiscriminate use of mineral oil as a laxative. On the other hand, it is to be noted that approximately one-third to one-half of the vitamin served its purpose and that, moreover, a varied diet supplies, in addition to carotene, vitamin A which apparently is better utilized in the presence of mineral oil. Furthermore, although relatively large doses of the corn were not tested, it seems generally possible in this type of experiment to overcome the effect of the mineral oil by the inclusion of extra vitamin A in the diet.

SUMMARY

1. The administration to albino rats of mineral oil separately from restricted doses of yellow corn interferes considerably with the assimilation of the vitamin A factor in the corn.

2. A discussion of the different responses produced by the vitamin A factors in butter fat and yellow corn is presented.

[2] More recently, Shrewsbury and Kraybill ('33) have concluded that: "The carotene of butter may account for an appreciable amount of its vitamin A activity."

ADDENDUM

The reader's attention is directed to a recent publication by H. S. Mitchell ('33), "Influence of mineral oil on assimilation of vitamin A from spinach," Proc. Soc. Exp. Biol. and Med., vol. 31, p. 231. Mitchell's results with spinach are very similar to the writer's with yellow corn and are in accord with the thesis discussed in this paper that mineral oil in the gut exerts a much more marked influence on the assimilation of carotene than on that of vitamin A.

CORRECTION

In the first paper of this series (Jackson, '31), group VII of chart 5 on page 181 should bear the legend: 5E, 4T, 1B—comparable to similar legends accompanying the other groups.

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THE EFFECT OF MINERAL OIL ADMINISTRATION UPON THE NUTRITIONAL ECONOMY OF FAT-SOLUBLE VITAMINS

III. STUDIES WITH VITAMIN D OF IRRADIATED ERGOSTEROL[1]

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Mineral oil in the gut may compete with natural fat as a solvent for fat-soluble substances. The fraction retained by the mineral oil which is itself not absorbed to any appreciable degree can be expected to escape the body. These considerations have been shown to have an important bearing relative to the effect of the ingestion of mineral oil upon the bodily economy of the fat-soluble vitamins. In addition to a considerable literature dealing with vitamin A in this regard, a few similar contributions on vitamin D have appeared.

Györky ('27) pointed out that olive oil should prove better than paraffin oil as a diluent for irradiated ergosterol because olive oil unlike mineral oil is absorbed. Nevertheless, Dutcher, Ely and Honeywell ('27) reported that cod liver oil diluted with mineral oil promotes calcification in rachitic rats to the same degree as an equal amount of cod liver oil without the mineral oil. The daily dose of cod liver oil was 4 drops (118 mg); of the cod liver oil-mineral oil mixture, 15 drops containing 4 drops of the former. Indices of the alleviation of rickets were bone ash percentage and the 'line' test.

On the other hand, Hawk, Levine, Stuckey and Oser ('29) state:

[1] The expenses of this investigation were defrayed from the income of the Mineral Oil Fellowship, Yale University, 1929–1930.

Using the 'Line Test' and X-ray as criteria, it was demonstrated, both in preventive and curative experiments, that mineral oil also interfered with the utilization of vitamin D in cod liver oil. Approximately three to four times as much vitamin D was required to produce recalcification when mineral oil, as a diluent, was substituted for cottonseed oil.

The different results obtained by Dutcher, Ely and Honeywell and Hawk, Levine, Stuckey and Oser are apparently to be explained on the basis of the smaller dosage (2 to 12 mg.) of cod liver oil employed by the latter authors.

The experiments cited above deal with the effect of mineral oil given in an intimate mixture with the material containing the vitamin D. The following study was undertaken to determine the influence of mineral oil administered separately from the vitamin D. The experiments were thus designed to simulate the customary human practice in the use of mineral oil.

EXPERIMENTAL PART

Animals, diet and feeding technic. Albino rats[2] approximately 4 weeks of age and weighing from 50 to 60 gm. were confined four to a cage and fed a high calcium-low phosphorus rachitogenic diet for 24 days. Growth records were kept during this time. The animals were then recaged singly and divided into groups which were subjected to various regimes for an experimental period of 15 days. Body-weight and food-consumption records were kept for this period. Starvation was thereby demonstrated not to be a factor in the healing of the induced rickets. Fat was sometimes incorporated to make the diet more nearly like the average food of man, but the amount was restricted to avoid a correction of the abnormal calcium-phosphorus ratio in the gut (compare Zucker and Barnett, '22–'23). All diets were fed ad lib.

The irradiated ergosterol[3] preparations employed were made up in and diluted with corn oil[4] to give the proper dos-

[2] The animals employed in experiment 1 were reared on Sherman's diet B; those in experiment 2 on a calf meal ration (Maynard, '30). Both diets were somewhat modified for our use.

[3] The writer wishes to thank Dr. Charles N. Frey, of the Fleischmann Laboratories, New York, for the ergosterol preparations.

[4] Mazola.

ages in 20-mg. drops which were administered daily at 11 to 12 A.M. by means of a calibrated medicine dropper. The mineral oil, of two kinds, no. 1 and no. 2, previously described (Jackson, '31) was given daily in three equal portions at 8 to 9 A.M., 2 to 3 P.M. and 5 to 6 P.M. by means of an insulin syringe.

At the conclusion of the experimental period, the animals were killed and the femora and either the tibiae or the radii and ulnae were removed. The femora from each rat were carefully freed of adhering tissue, and the ash determined as per cent of dry fat-free substance according to the directions of Shohl and Bennett ('27). The other bones were subjected to the 'line' test with silver nitrate (McCollum, Simmonds, Shipley and Park, '22, and Coward, '28) or were decalcified in Müller's fluid, sectioned and stained with hematoxylin and eosin for histological examination.

Experiment 1

Both male and female animals were employed. Steenbock's diet 2965 (Steenbock and Black, '25) plus 10 per cent by weight of hydrogenated vegetable oil[5] was fed throughout. The average gain in body weight for the 15-day experimental period was 5 gm. The amount (0.005 mg.) of viosterol employed in the mineral oil tests was in excess of the minimum required to induce a considerable recalcification. The total daily dosage of mineral oil was 0.5 cc.

Experiment 2

Only male albino rats were employed. Diet 2965 was fed for the initial 24 days, and then diet 2965 with 5 per cent of added hydrogenated vegetable oil for the experimental period of 15 days. The animals on the average just maintained their body weights during this latter period. To exclude the effect of laboratory light (compare Dutcher and Honeywell, '29), the animals were maintained for the entire 39 days behind a

[5] Crisco.

black curtain. The irradiated ergosterol preparation was a different one than that used in experiment 1; the amount (0.001 mg.) employed in the mineral oil tests was insufficient to promote more than a partial calcification. The total daily dosage of mineral oil was 0.3 cc.

DISCUSSION OF RESULTS

The values for bone ash presented in table 1, experiment 1, exhibit no significant differences for groups II to V, although

TABLE 1

The calcifying effect of irradiated ergosterol with and without mineral oil. Experiment 1

GROUP NO.	NUMBER OF ANIMALS	DAILY DOSE OF IRRADIATED ERGOSTEROL	DAILY DOSE OF MINERAL OIL	AVERAGE DAILY FOOD CONSUMPTION	BONE ASH; ON THE BASIS OF DRY FAT-FREE SUBSTANCE		
					Lowest value	Highest value	Average value
		mg.	*cc.*	*gm.*	*Per cent*	*Per cent*	*Per cent*
I	6	None	None	6.2	26.8	36.9	33.1
II	7	0.001	None	5.8	34.2	46.0	40.0
III	8	0.005	None	5.2	36.3	43.7	40.0
IV	8	0.005	0.5 of no. 1	5.2	38.4	45.0	42.0
V	8	0.005	0.5 of no. 2	5.2	37.4	44.6	41.5

the average figure for group I receiving no irradiated ergosterol is considerably lower—33.1 per cent. 'Line' tests performed on the radii and ulnae revealed a similar relation. The animals of group I were shown to have marked rickets but those of the remaining groups were found to present pictures of advanced healing. Thus, administration of 0.5 cc. of mineral oil daily to the animals was without apparent effect on the utilization of the vitamin D; however, the dosage of the latter was at least five times the amount required to induce the maximum effects observed.

In experiment 2 a different specimen of irradiated ergosterol was examined under somewhat altered conditions as previously described. One of these conditions was that of administering a sub-marginal dose of irradiated ergosterol; another, that of keeping the animals in the dark. The average value for the bone ash of the femora of the negative control animals (group I) is 25.3 per cent. The other groups exhibit a progressive elevation of the bone ash with increase in the dosage of the irradiated ergosterol. The administration of mineral oil is without any apparent effect on calcification as

TABLE 2

The calcifying effect of irradiated ergosterol with and without mineral oil. Experiment 2

GROUP NO.	NUMBER OF ANIMALS	DAILY DOSE OF IRRADIATED ERGOSTEROL	DAILY DOSE OF MINERAL OIL	AVERAGE DAILY FOOD CONSUMPTION	BONE ASH; ON THE BASIS OF DRY FAT-FREE SUBSTANCE		
					Lowest value	Highest value	Average value
		mg.	*cc.*	*gm.*	*Per cent*	*Per cent*	*Per cent*
I	9	None	None	4.8	24.2	28.1	25.3
II	8	0.0002	None	5.5	28.6	33.5	30.8
III	10	0.001	None	5.0	29.7	37.6	32.9
IV	10	0.001	0.3 of no. 1	5.3	28.4	36.5	32.9
V	10	0.001	0.3 of no. 2	5.4	29.4	35.8	33.1
VI	8	0.005	None	5.7	32.2	38.4	35.3

measured by ash determination, all the averages for groups III, IV and V being very close to 33 per cent. Sections of the tibiae were stained with hematoxylin and eosin and examined under the microscope.[6] The negative controls (group I) uniformly revealed the development of severe rickets without healing. Half of the animals of group II showed no healing and half, from 1 plus to 2 plus healing. Five of the seven animals of group VI showed a nearly normal structure. The

[6] The writer expresses his appreciation to Miss Deborah Jackson of the Department of Pediatrics of the Johns Hopkins Hospital for the examination of these sections.

members of groups III, IV and V were intermediate in this respect; approximately half in each series showed no healing and half from 2 plus to 3 plus healing.

These results show no adverse effect upon calcification when mineral oil is administered separately from the oil bearing the vitamin D. Furthermore the comparable nutritive state of the experimental animals of the mineral oil groups and the corresponding control group in each experiment is indicated by the nearly identical food consumptions.

SUMMARY

Mineral oil administered separately from irradiated ergosterol was not found to interfere with the utilization of vitamin D.

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THE CHEMISTRY OF THE BLOOD OF NORMAL CHICKENS

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In an investigation of the effect of the Rous sarcoma no. 1 upon the chemistry of the blood of hens (Roe and Dyer, '30, '31; Dyer and Roe, '32), it was necessary to establish normal values by determining the constituents of the blood of control hens. Since the literature contains very little information upon the blood chemistry of hens and hens are used rather extensively in certain types of experimental work, the authors feel that a published summary of their findings will be of value. In this paper we are reporting a summary of chemical analyses of normal hens' blood which includes values for twenty-three constituents. The reference to the method of analysis used, the number of hens, the number of determinations, the lowest and the highest individual values, and the average value for each determination are given.

The hens used were of the Barred Plymouth Rock strain and were obtained from market stock. Their average weight was approximately 2 kg. They were kept on a diet of water and 'scratch feed,' which is a commercial preparation of cracked corn, wheat, barley, buckwheat, milo maize, and sunflower seed. All analyses were performed after the hens had been fasted for approximately 24 hours.

The methods of analysis were thoroughly tested and sufficient analytical skill to obtain reproducible results was developed before undertaking the study of a blood constituent. Because of the undesirable influence of excessive bleeding,

The chemistry of the blood of normal hens (values are expressed as milligrams per 100 cc. of whole blood unless otherwise indicated)

CONSTITUENT	NUMBER OF HENS	NUMBER OF DETERMINATIONS	VALUES			METHOD OF ANALYSIS
			Lowest	Highest	Average	
Non-protein nitrogen	13	13	28	47	36	Dupray's modification of Folin and Wu ('27)
Urea	4	10	2.1	5.4	3.3	Leiboff and Kahn ('29)
Uric acid	9	9	2.6	7.5	4.2	Folin's direct ('22)
Creatinine	17	17	0.8	1.5	1.3	Folin and Wu ('19)
Albumins of plasma	8	22	870	1,970	1480	Greenberg ('29)
Globulins of plasma	8	22	1460	2,770	1830	Greenberg ('29)
Hemoglobin	5	5	6300	11,000	8800	Van Slyke and Stadie ('21)
Sugar	19	19	127	190	157	Rothberg-Evans modification of Folin and Wu ('23)
CO_2-combining power[1]	10	10	24	53	42	Van Slyke ('17)
pH	14	72	7.22	7.45	7.36	Hastings and Sendroy ('24)
Fatty acids	8	14	411	1,560	847	Bloor, Pelkan and Allen ('22)
Carotin of plasma	6	10	0.29	2.0	0.99	Connor ('28)
Cholesterol	15	15	114	244	168	Bloor ('16)
Phospholipids (as lecithin)	10	15	231	580	420	Modification of Fiske and Subbarow ('25)
Phosphorus (inorganic of serum)	6	6	2.7	5.9	4.6	Modification of Fiske and Subbarow ('25)
Phosphorus (total of plasma)	4	19	10	19	13	Modification of Fiske and Subbarow ('25)
Phosphorus (total)	9	38	92	132	105	Modification of Fiske and Subbarow ('25)
Chlorides as NaCl	14	14	517	644	563	Modified Whitehorn ('21)
Sodium of serum	10	18	279	385	345	Kramer and Gittleman ('24)
Potassium of serum	9	17	19	27	22	Breh and Gaebler ('30)
Potassium	7	13	130	192	164	Breh and Gaebler ('30)
Calcium of serum	4	4	12.4	12.8	12.7	Roe and Kahn ('29)
Magnesium of serum	5	12	1.6	3.0	2.4	Eichholtz and Berg modification of Yoshimatsu ('30)
Glutathione (reduced)	9	14	60	94	75	Hess ('29)
Bilirubin of serum	4	4	Trace	Trace	Trace	Van den Bergh ('16)

[1] Volumes per cent.

it was decided not to make duplicate determinations of all constituents, and accordingly duplicate determinations were made only upon those constituents for which we felt the method of analysis was not sufficiently accurate to give entirely satisfactory results by single determinations. Duplicate determinations were made in estimating the non-protein nitrogen, fatty acids, total phosphorus, phospholipids, calcium, magnesium, sodium, potassium, albumins and globulins. In view of the fact that a great number of determinations was made we believe that our findings represent true values for chickens' blood.

Values for some of the constituents in our summary have been reported by Hayden and Fish ('28), Hughes, Titus and Smits ('27), Haam and Stöhr ('30), Holy ('30), Moravek ('32, '32 a), and Kobliha ('30). The reports of these authors are in agreement with our findings when the variations due to the methods of analysis used are taken into consideration.

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THE RELATION OF FOOD TO ALIMENTARY FILL IN THE ALBINO RAT [1]

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It is generally recognized that the basal heat production of animals differing in size is not a constant, in relation to their respective body weights; consequently diverse conclusions have arisen as to the relationship of body size to metabolism, and various units of reference for basal metabolism have been suggested.

Inasmuch as any one of these units ultimately involves the weight of the animal, it is immaterial, for the purpose of the present consideration, whether heat production is referred to body weight directly, or to some functional power of the body weight, or to derived units, such as computed body surface, or active protoplasmic mass. It is of interest, however, to consider whether there is not a more significant measure of body weight than the gross weight, as a unit of reference for metabolism, or as a basis for the computation or derivation of units of reference.

The degree of fatness of an animal is one factor which influences the extent of the body surface, and of the body weight—in a sense 'diluting' the active body tissue participating in metabolism (Moulton, '16).

Another factor, which was considered by Rubner (1883), is the content of the alimentary tract, which represents a considerable and a varying proportion of the body weight, but

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does not constitute a direct or an immediate proportionate cause or source of heat production.

The present paper presents certain observed relationships between the quantities of the food eaten and of the alimentary fill, and calls attention to their significance in the statement of results of metabolic research.

EXPERIMENTAL

This study of fill was conducted on a separate group of animals in connection with a concurrent investigation of the influence of the plane of nutrition on the heat production and on the specific dynamic effects of nutrients, the two groups of animals, which were albino rats weighing about 100 gm. each, being fed and managed alike. The dietary treatment is given in table 1.

The rats were confined in individual cages with false screen bottoms to lessen coprophagy. The daily food was given in two equal portions, at 8 a.m. and 4:30 p.m.

In the energy metabolism studies the following procedure was followed: With all except the fasting rats the metabolism experiments were conducted after the subjects had been not less than 3 days on a specific dietary treatment. The experiments were started immediately after the morning feed allotment had been consumed—which was usually within an hour after feeding. With fasting rats a maintenance allowance of a complete diet was fed for 3 or more days. The animals were then used for the metabolism experiment 24 hours after receiving the last half-daily portion of feed.

In the concurrent study of fill the rats were killed after the same time following feeding as elapsed with the rats used for metabolism studies before respiration measurements were begun. The rats were killed by asphyxiation, and were weighed on a large analytical balance. The alimentary tracts were removed and emptied, and the contents were placed in weighing bottles, and weighed. At least five individuals were used for each dietary treatment. The results are presented in table 1.

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TABLE 1

The relation of the quantity of feed to the quantity of the contents of the alimentary tract

GROUP	NUMBER OF ANIMALS	DAILY DIETARY TREATMENT	WEIGHT OF CONTENTS OF ALIMENTARY TRACT EXPRESSED AS PER CENT OF GROSS BODY WEIGHT						
			Individual determinations						Mean
1	5	Fasting	2.39	2.73	3.02	3.36	3.87	....	3.07 ± 0.18
2	5	1.56 gm. olive oil	4.70	5.05	5.68	5.77	5.88	....	5.42 ± 0.15
3	5	3.80 gm. casein	4.84	5.39	6.52	6.92	7.02	....	6.14 ± 0.29
4	6	3.92 gm. corn starch	4.63	5.69	6.66	7.49	7.58	8.14	6.70 ± 0.35
5	5	4.00 gm. calf meal [1]	8.88	9.21	9.26	9.69	10.07	....	9.42 ± 0.14
6	5	5.50 gm. calf meal	9.57	9.58	9.87	10.47	10.63	....	10.02 ± 0.15
7	5	5.50 gm. calf meal + 1 gm. olive oil	9.04	9.28	9.44	9.45	9.86	....	9.41 ± 0.09
8	5	5.50 gm. calf meal + 2.0 gm. casein	8.56	9.83	10.20	10.53	10.84	....	9.99 ± 0.29
9	5	5.50 gm. calf meal + 2.2 gm. corn starch	9.89	9.99	10.18	10.83	10.88	....	10.35 ± 0.14
10	5	8.00 gm. calf meal	8.52	11.24	11.52	11.63	13.38	....	11.26 ± 0.53

[1] The calf meal diet was composed of 300 parts linseed oil meal, 400 parts corn meal, 200 parts ground malted barley, 440 parts red dog flour, 240 parts dried skim milk, 300 parts oat flour, 60 parts soluble blood meal, 20 parts salt, 20 parts ground limestone and 20 parts steamed bone meal.

DISCUSSION

The energy requirement for the maintenance of a 100-gm. rat was approximately satisfied by the olive oil, starch and casein fed to groups 2, 3 and 4, and by the 5.5 gm. allowance of calf meal fed to group 6. Groups 7, 8, 9 and 10 received food in excess of the amounts required for maintenance.

The lowest proportion of fill, 3.07 per cent, was obtained from the fasting rats. For the groups fed the approximate requirements of maintenance—the fill with olive oil was 5.42 per cent; with casein, and with starch, 6.14 and 6.70 per cent, respectively; and with 5.5 gm. of calf meal, 10.02 per cent. The fill with 4 gm. of calf meal was considerably greater than with almost equal quantities of starch or casein, and was only slightly less than with 5.5 gm. of calf meal. These figures indicate that the proportion of fill is influenced both by the quantity and by the character of the feed.

The addition of casein or starch to the maintenance diet of calf meal did not result in a significant increase in fill. The fill on the maintenance diet of calf meal plus olive oil was slightly lower than that for the same quantity of calf meal alone—probably because of a laxative effect of the olive oil.

An increase in the quantity of calf meal from 5.5 gm. to 8 gm., that is, a 45 per cent increase in the diet, resulted in a 12 per cent increase in fill, and affected the weight of the animal by only 1.2 per cent. In other words at the supermaintenance levels the influence of the character of the diet on the fill was much less pronounced than at the maintenance levels. This was, of course, partly due to the fact that the greater part of all the supermaintenance rations here considered consisted of the same calf meal, whereas the maintenance rations differed much in character. The fact, however, that the addition of the various single supplements, and of more calf meal, to the maintenance ration of calf meal did not result in any considerable increase in fill signifies that with increase in food, of any kind, there is a proportionately greater increase in the rate of passage of food residues through the alimentary tract.

The foregoing observations are especially important in relation to the comparison of the rates of metabolism of animals on different dietary treatments. From unpublished results of metabolism experiments the authors have computed the daily heat production of fasting rats, and of rats fed a maintenance quantity of a complete diet, i.e., 5.5 gm. per day. When the heat production is expressed per 100 gm. of empty body weight, the heat increment resulting from the 5.5 gm. of feed is 3360 small calories. If no correction is made for the alimentary fill the heat increment is 2808 calories. A difference of the same order obviously results if other units of reference for metabolism are used, the derivation of which involves the body weight; for instance, computed body surface.

The individual variation in the fill of animals on the same dietary treatment was slight.

In harmony with this finding Trowbridge, Moulton and Haigh ('15) observed average variations in the fill of mature beef cattle of only 2 to 4 per cent of the live weight, and suggest that the fill can be disregarded in many cases with those animals. However, it is more logical to relate the metabolism to a body weight which represents only body tissue; and such procedure is important for a close comparison of the metabolism of animals subjected to different dietary treatments.

SUMMARY

The quantity of the alimentary fill, expressed as a percentage of the live weight, has been determined with ten groups of albino rats, one group being fasted, the other groups being given different dietary treatments.

The ratio of fill to gross body weight was found to be somewhat affected by both the kind and amount of feed, such effects being more prominent at the maintenance level than at higher planes of nutrition.

The results indicate that with increase in food there is a proportionately greater increase in the rate of passage of food residues through the alimentary tract.

The alimentary fill of rats which received food was greater than that of fasting rats by 2.4 to 8.2 per cent of the gross body weight, in accord with the dietary treatment.

It is therefore important to consider alimentary fill when comparing the metabolism of animals subjected to different dietary treatments.

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A COMPARISON OF EXTRACTION METHODS USED FOR VITAMIN A DEPLETION DIETS FOR RATS

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INTRODUCTION

The present-day worker in the field of vitamin A assay is confronted with many problems in determining the technic to use in his work. Osborne and Mendel ('13) and McCollum and Davis ('13) called attention to the varying length of time that different animals of the same species continued to grow upon diets lacking in vitamin A. Chick ('20) stated that in her work extending over several years there was a great deal of unexplained irregularity in the behavior of the stock rats when placed upon diets deprived of fat-soluble vitamins. Sherman and Boynton ('25) and Sherman and Storm ('25) found a wide variation in the survival period. The same observation was made by Sherman and Cammack ('26) the following year. The recent work of Sampson and Korenchevsky ('32) showed that there was a wide variation in the time required for rats placed upon a vitamin A-free diet to develop either xerophthalmia or loss of appetite.

Bacharach ('33) in his investigation into vitamin A-free basal diets said that many animals will continue growing on a basal diet for months with no evident signs of avitaminosis, although some animals on the same diet reach a plateau in 8 to 10 weeks. Other animals appear to stop growing for as long as 14 days and then begin to grow again for 4 weeks or more. He further stated that, "The literature abounds in

graphs showing animals that have been considered 'runout' when their growth has only slackened, without actually ceasing, for a few days."

There is no standardized method of extraction of the casein offered at present. The solvent and the method and length of extraction reported by different workers varies greatly. Coward, Key and Morgan ('28) discussed at length the relative potencies of the extracts of casein made by several methods and solvents. They found that the factor causing growth resumption is removed from casein only with difficulty. Insufficient purification of the basal diet was, in the opinion of Drummond and Coward ('20), responsible for many variations. They stated that their own experience had given many examples of the fact that the presence of very small amounts of the vitamin A factor in an insufficiently purified basal diet may confuse the issue of the experiment. An analysis of the technic of representative workers showed a wide variation in the solvent and method used. The time of extraction has varied from heating 1 week in shallow pans to 10 days' continuous extraction with alcohol in a percolator.

The problem of the purification of the basal diet is of considerable importance. The presence of even small amounts of vitamin A in this diet will result in a prolonged depletion period and will cause a higher vitamin value to be assigned to the food being assayed.

Most of the published work concerning vitamin A has appeared in papers dealing with the detection or estimation of this vitamin, while few studies of the method of purification of the vitamin A-free diet have been reported.

The purpose of this study was to compare the effect of different methods of extraction with 95 per cent alcohol on the removal of vitamin A from the casein used in basal diets for rats.

PROCEDURE

The rats used in this study were of the albino or pied variety of known nutritional history from the stock colony. The mothers of these rats had been reared on a diet consisting of:

	Per cent
Whole wheat ground fine	30
Yellow cornmeal	60
Linseed meal	4
Bran	4
Sodium chloride (NaCl)	1
Tri-calcium phosphate ($Ca_3(PO_4)_2$	1

Cod liver oil ($\frac{1}{2}$ cc. per rat) was mixed with enough of the above for 1 day's feeding and water added to make a stiff paste. The diet was supplemented by milk and raw liver. The milk was fed ad libitum. The liver was ground and 2 to 3 gm. fed per rat per week.

One hundred and thirty rats were placed upon six different experimental diets. The general plan of the experimental work was to feed a diet nutritionally adequate except for vitamin A.

The vitamin A-free or depletion diet, a modification of Rowntree's ('29) basal diet, consisted of:

Material	*Grams*	*Remarks*
Casein	18	Commercial casein (crude Argentine[1]), extracted with hot alcohol (95 per cent by volume).
Yeast	10	Pure dehydrated yeast.[2]
Salt mixture	4	McCollum's no. 185.
Cornstarch	68	Commercial cornstarch (unpurified).
Viosterol	0.05	To the above was added 0.05 cc. of 2 per cent potassium iodide (KI).

[1] Atterbury Brothers, 145 Nassau Street, New York, N. Y.

[2] The Northwestern Yeast Company, Chicago, Illinois.

The animals were weighed weekly for the first 4 weeks, twice weekly thereafter until a stationary weight was reached, and then daily. They were examined for any outward signs of vitamin A depletion. A record of food consumption was kept for each cage of rats. At the end of the experimental period the rats were examined thoroughly, autopsied and a record made of their condition.

The casein used in diet I was extracted with hot alcohol for 16 continuous hours. The extraction was a modification of the Sperry method ('26) as used by Rowntree ('29). To 200 gm. of fine-grained, air-dry, crude Argentine casein were added 500 cc. of boiling 95 per cent alcohol and the mixture was allowed to stand overnight to prevent caking. The following day the material was filtered by suction in a Büchner filter. The casein was placed in a cheesecloth bag inside an enamel percolator. The alcohol was placed in a 2-liter Pyrex flask, and kept at a slow rolling boil. A round-bottom 2-liter Pyrex flask fitted tightly into the top of the percolator, and served as a condenser. At the end of the extraction period the hot casein was filtered rapidly by suction in a Büchner filter, air dried, and then heated for 1 week in shallow pans in an electric oven at 105° to 110°C. The material was stirred thoroughly each day.

In diet II the method of extraction of the casein was a combination of the Sperry method as described for diet I, and Sherman's method ('31). The same apparatus was used. The casein (200 gm.) was treated in three successive 1-hour extraction periods, using 500 cc. of fresh alcohol for each extraction. Finally, the casein was dried in the air at room temperature.

The method used for the extraction of the casein fed to rats on diet III was the same as that used for diet I with the exception that 1500 cc. of 95 per cent alcohol were used to 200 gm. of casein.

An entirely different technic was followed for the extraction of the casein for diets IV and V. The Sherman ('31) method was used. Two hundred grams of casein (without previous soaking) were placed in a 2-liter Pyrex flask with 500 cc. of 95 per cent alcohol. This was boiled 1 hour under a reflux condenser on an electric plate. The contents of the flask were transferred rapidly without cooling to the Büchner filter and the hot alcohol removed by suction. The casein was returned to the flask, 500 cc. of fresh 95 per cent alcohol added, and the extraction continued for another hour. The process

was repeated a third time. The casein was dried in the air at room temperature.

The majority of the workers in the field do not extract the yeast to free it of vitamin A, believing that the amount of A is too small to be a determining factor. However, Sherman and Smith ('31) recommend that each laboratory carefully test the yeast that it uses in vitamin A work. Osborne and Mendel ('20) state that heating yeast overnight in an electric oven at 110°C. and then boiling it three successive times with

TABLE 1

Summary of methods of extractions of casein with 95 per cent alcohol

DIET	METHOD		AMOUNT OF		LENGTH OF EXTRACTION	REMARKS
	Direct	Indirect	Casein	Alcohol		
			gm.	*cc.*	*hours*	
I		Casein in bag in percolator	200	500	16	Continuous
II		Casein in bag in percolator	200	1500	3	Successive, fresh alcohol added
III		Casein in bag in percolator	200	1500	16	Continuous
IV	Casein in flask		200	1500	3	Successive, fresh alcohol added
V	Casein in flask		200	1500	3	Successive, fresh alcohol added
VI		Casein in bag in percolator	200	1500	48	Continuous

absolute alcohol under a reflux condenser for 1 hour does not affect the potency of the vitamin B and G content.

In diet V both the casein and the yeast were extracted by the direct method used for the extraction of the casein in diet IV.

A continuous 48-hour extraction was made on the casein used in diet VI by the indirect method used in diet III.

Table 1 gives a summary of the extraction methods, proportion of ingredients, and length of time of extraction used in the six different diets.

The young rats received the regular stock diet from the time of weaning until they were placed on the various diets. Each litter was kept in a small round raised-bottom screen cage which prevented coprophagy. When they approached maturity the males and females were separated. The experimental period was 10 weeks.

RESULTS

In this paper we have presented data on the effectiveness of different methods of extraction for the removal of vitamin A from casein used in vitamin A-depletion diets.

TABLE 2

Comparison of initial age and weight with final weight

DIET	NUMBER OF		AGE IN DAYS		WEIGHT IN GRAMS			
			Initial		Initial		Final	
	Rats	Litters	Range	Average	Range	Average	Range	Average
I	37	6	24–28	26.2	33–43	38.5	150–228	185.5
II	25	5	22–23	22.2	31–39	35.3	134–222	174.5
III	15	6	24–28	26.4	27–44	34.8	97–149	123.2
IV	17	6	21–24	22.7	29–39	33.1	67–154	121.1
V	18	6	21–25	23.2	27–38	32.0	104–149	121.6
VI	18	6	22–28	25.8	27–38	34.6	93–185	145.1

The results from diets I and III have been compared to show the effect of varying the amount of alcohol; diets II and IV to determine the effect of different methods of extraction; diets III and VI to note the effect of a variation in the time of extraction; and diets IV to V to determine whether any appreciable amount of vitamin A could be removed from the yeast. Table 2 shows that there was very little variation in the initial ages and weights of the rats in the groups which were compared.

Diets I and III were the same in all respects except in the amount of alcohol used for the extraction of the casein. These two diets were studied to determine the effect of a different amount of alcohol on the removal of vitamin A from casein. Three times as much alcohol (1500 cc.) was used

in the extraction of the casein (200 gm.) for diet III as was used for diet I (500 cc.). Both extractions were made by the Sperry method for 16 continuous hours, placing the casein in a bag inside a percolator. Since all animals were bred on the same stock ration, and the average initial age was approximately equal, the principal conditions for the storage of vitamin A were constant. The results obtained from feeding these two diets were very different. No abnormal conditions were observed among the rats on diet I at autopsy. Ninety-four per cent of the rats on diet III developed lung infection, while 80 per cent showed an abnormal condition of the spleen. Thatcher and Sure ('32) stated that atrophy of the spleen

TABLE 3

Average gains per rat per week for experimental period

DIET	NUMBER OF RATS	WEEK										AVERAGE
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
I	37	26.5	29.0	19.1	25.1	7.5	6.2	7.1	8.9	10.3	7.3	14.70
II	25	18.9	25.1	22.5	22.4	21.2	15.6	8.4	7.8	− 0.4	− 2.3	13.56
III	15	24.7	23.2	17.3	16.0	17.8	2.9	5.8	−2.9	− 7.0	− 9.4	8.84
IV	17	20.9	24.3	30.7	15.0	19.3	10.2	0.0	−9.9	−13.9	−14 0	8.26
V	18	18.4	24.6	22.6	23.2	16.9	10.6	1.0	−8.0	−11.5	−20.0	7.78
VI	18	18.3	24.9	19.5	22.4	15.0	9.3	4.8	−0.15	− 1.1	− 2.5	11.04

was a common finding in their vitamin A work. Nearly one-half gave evidence of an abnormal kidney condition, and about one-fifth showed abnormalities of the eye, ear and liver. The rats on diet I made a 381 per cent gain as compared with 254 per cent for those on diet III (table 3). The average weekly gain per rat was nearly twice as much on diet I as on diet III. The first break in weight on the two diets was at the same time (third week), but the final decline after which no further gain was made occurred in the seventh week in the rats on diet III, and in the tenth week on diet I. The daily food consumption was the same for the rats on both diets. Sixty-seven per cent of the animals on diet I showed a stationary or lowered weight for 3 successive days with a sub-

sequent gain, whereas only 53 per cent of the animals on diet III exhibited this phenomena. Bacharach ('33) stated that many animals on a vitamin A-free diet reached a plateau or showed a decline in weight for a few days and then continued to grow again for some time. Many workers in the field of vitamin A assay have used stationary weight for 3 successive days as the criteria for depletion. All the rats on both diets were alive at the end of the experimental period. The results obtained by feeding these two diets to rats indicated that the large amount of alcohol (1500 cc.) was more effective in the removal of vitamin A from casein (200 gm.) than the small amount (500 cc.).

Diets II and IV were compared to determine the effect of the method of extraction on the removal of vitamin A from casein. The casein in diet II was extracted by the Sperry method while Sherman's method was employed for the extraction in diet IV. The extraction was carried on with the same amount of alcohol (1500 cc.) and for the same length of time, 3 successive hours, with fresh alcohol each hour. No abnormal conditions were noticed among the rats on diet II. Fifty-eight per cent of the rats on diet IV developed a lung infection, while about one-half showed abnormalities of the ear and liver. In a few cases the pupil of the eye became white and opaque, resulting in apparent blindness. In some cases where inflammation of the lids was noticed among the rats in this group, the condition which is generally considered a sign of vitamin A depletion seemed to be due to colds and not to xerophthalmia. At various times rats from the stock colony suffering from colds have shown a more evident inflammation around the eye than was observed in this group of rats. One wonders whether some of the eye conditions which have been reported as xerophthalmia have been due merely to colds. It is difficult to distinguish between actual xerophthalmia in rats and the condition of the eyes commonly found in colds. The determination of the abnormality of the liver from general observation of a spotted condition is very subjective, and should be followed by a microscopical study if it is to be

considered a significant factor in depletion work. Slightly over one-fourth of the rats on diet IV developed an abnormal condition in the kidney, alimentary tract and nasal passages. Seventeen per cent of these animals had abscesses in the posterior portion of the tongue, while 5 per cent showed an abnormal eye condition and bloody urine. The rats on diet II made a significantly better per cent gain (394 per cent) for the entire experimental period than those on diet IV (239 per cent) (table 3). The average weekly gain was 13.92 gm. for the rats on diet II and 8.26 gm. for the rats on diet IV. The average daily food consumption for the two groups of rats was almost identical, varying only 0.5 gm. The first decline in growth for the rats on diet II was in the third week, and for diet IV in the fourth week. The beginning of the continuous decline occurred earlier in rats on diet II (third week) than in the rats on diet IV (sixth week). The weight of the rats on diet II held quite constant until the sixth week when an average loss of 15 gm. per rat occurred. Forty-eight per cent of the rats on diet II attained plateaus and later showed gains, whereas only 11 per cent did so on diet IV, indicating more uniform results for the latter diet. The lowest per cent of plateaus with subsequent growth was found in the rats on diet IV. Mortality among the rats on diet IV was 29 per cent. The method of boiling the alcohol and casein in the flask seemed to remove more vitamin A than the method of placing the casein in a bag in a percolator.

A comparison was made of diets IV and V to ascertain whether the yeast used in the six diets contained any appreciable amount of vitamin A which might confuse the results of an assay. The casein alone was extracted in diet IV, while both casein and yeast were extracted in diet V. The Sherman method was used for all extractions in these diets. There was approximately six times as great an incidence of abnormal eye condition in the rats on diet V. Forty-seven per cent of the rats on both diets developed pus in the ears. There were no instances of abscesses on the posterior portion of the tongue or of bloody nose and paws in rats on diet V, while

those on diet IV showed 17 and 27 per cent respectively of these conditions. A slightly higher incidence of lung, spleen, and kidney abnormalities appeared in the rats on diet V, while there was a slightly greater per cent of liver changes in the rats on diet IV. There was a marked difference in the condition of the alimentary tract at autopsy; the rats on diet V showed a higher per cent and greater severity of changes. A day or two before death the abdomens of the rats on diet V became swollen and hard, although excretion was normal. The tract was greatly distended with gas, somewhat darkened and in a few cases contained blood. Turner and Loew ('31), in their work on the infection of the accessory sinuses and upper respiratory tract in avitaminosis of rats, stated that at necropsy 40 per cent of the animals deprived of vitamin A showed a ballooning of the intestines and marked gastro-enteritis. While no abnormal condition of the urine was observed in the rats on diet V, 5 per cent of the rats on diet IV developed a bloody urine. Neither the average weekly gain nor the total per cent gain for the experimental period showed any significant variation in the rats on these two diets, which indicated that no appreciable destruction of vitamins B and G had resulted from the alcohol extraction of the yeast. These results seem to confirm the observations of Osborne and Mendel ('20) that the extraction of yeast with hot alcohol does not remove vitamins B and G. One-third of the rats on diet V reached plateaus and later gained in weight, while only one-tenth of the group did so on diet IV. The mortality of the rats was twice as high on diet V as on diet IV. The food consumption for the two diets varied 1.43 gm., which showed that there was no appreciable amount of the appetite factor removed by the extraction of the yeast. Since the general condition of the rats fed the extracted yeast was somewhat worse than the condition of the rats fed the unextracted yeast, it would seem to indicate that the yeast used in this study did contain a small amount of vitamin A. It is interesting to note that even though there were many differences between diet V and the other diets, the rats on diet V showed in general the worst condition.

Diets III and VI were comparable in all respects except the length of time of extraction of the casein. The Sperry method of placing the casein in a bag inside a percolator and allowing the alcohol vapors to pass up through the casein was used for the extraction in both diets. Fifteen hundred cubic centimeters of alcohol were used for the extractions in both diets. The casein in diet III was extracted for 16 continuous hours, whereas the extraction for diet VI was continued for 48 hours. The results from the two diets were quite similar. There was approximately the same per cent occurrence of abnormalities in the eye, ear, tongue, kidney and urine in

TABLE 4

Average daily food consumption per rat

DIET	WEEK										AVERAGE
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	
I	11.3	15.7	19.2	16.4	16.9	15.4	14.9	16.4	18.8	18.3	16.33
II	9.4	14.2	18.4	18.2	18.8	18.5	17.8	18.8	17.8	18.1	17.00
III	11.4	17.8	19.4	19.1	19.6	18.9	18.8	17.8	10.9	9.5	16.32
IV	9.5	15.6	19.2	22.7	20.9	19.6	19.8	16.7	11.5	9.1	16.46
V	9.9	14.1	19.6	19.6	19.1	18.8	17.1	13.6	11.8	6.7	15.03
VI	11.8	14.5	19.7	17.0	16.8	18.0	17.5	12.5	12.9	12.0	15.17

the rats on the two diets. Diet III showed a higher incidence of cases of bloody nose and paws, lung infection, liver and spleen changes. Eleven per cent of the rats on diet VI developed abnormalities of the alimentary tract, whereas none was evident in the rats on diet III. There was a considerably greater per cent gain (319 per cent) on diet VI than on diet III (254 per cent). The rats on diet III gained 8.84 gm. weekly compared to a gain of 11.04 gm. for diet VI. The difference of 1.75 gm. in weekly food intake was insignificant. There were no deaths among the rats on either diet. The results obtained from feeding diets III and VI indicated that a 48-hour continuous extraction was no more effective in the removal of vitamin A from casein than was an extraction period one-third as long.

Average food consumption, as shown in table 4, was practically equal in all the groups of animals with a maximum variation of 1.7 gm. per rat per week. The uniform food consumption of the rats on the six different diets shows that the differences in the results obtained were due to a deficiency

TABLE 5

Summary of results

REMARKS		DIETS					
		I	II	III	IV	V	VI
Number of rats on diet		37	25	15	17	18	18
Average initial agee (days)		26.2	22.2	26.4	22.7	23.2	25.8
Average initial weight (grams)		38.5	35.3	34.8	33.1	32.0	34.6
First decline in weight (weeks)		3rd	3rd	3rd	4th	4th	3rd
First continuous decline (weeks)		10th	3rd	8th	6th	5th	5th
Plateaus of weight— later gained		67%	48%	53%	11%	331/3%	55%
Per cent gain—10-week period		381	394	254	239	243	319
Average weekly gain (grams)		14.7	13.56	8.84	8.26	7.78	11.04
Average daily food intake (grams)		16.33	17.0	16.32	16.46	15.03	15.17
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Deaths		0	0	0	29	61	0
Abnormal condition of	eye	0	0	20	5	33	16
	ear	0	0	20	47	47	22
	nose and paws	0	0	5	27	0	0
	tongue	0	0	0	17	0	0
	lungs	0	0	94	58	66	33
	liver	0	0	13	41	38	5
	spleen	0	0	80	35	47	55
	kidney	0	0	40	29	33	42
	tract	0	0	0	29	42	11
	urine	0	0	0	5	0	0

in the diets, and not to a lowered food intake. The fact that the stock ration used for the colony was so high in vitamin A may explain in part the prolonged depletion which occurred in this study.

CONCLUSIONS

A comparison of the effect of different methods of extraction with 95 per cent alcohol upon the removal of vitamin A

from the casein used in depletion diets for rats under the conditions of this experiment showed that:

1. The large amount of alcohol (1500 cc.) was more effective in the removal of vitamin A from casein (200 gm.) than the small amount (500 cc.).

2. The method of boiling the alcohol and casein in the flask seemed to remove more vitamin A than the method of placing the casein in a bag in a percolator.

3. A 48-hour continuous extraction with hot alcohol was no more effective in the removal of vitamin A from casein than a 16-hour continuous extraction period.

4. a) A comparison of extracted and unextracted yeast used in this study showed that the sample of yeast tested contained a small amount of vitamin A.

b) Observations of the growth and food consumption of the rats on the extracted and unextracted yeast indicated no appreciable loss of vitamins B and G in the yeast extracted with hot 95 per cent alcohol.

5. The extraction of both the casein and the yeast by the Sherman method resulted in the removal of the maximum amount of vitamin A from the basal diet.

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VITAMIN C IN DELICIOUS APPLES BEFORE AND AFTER STORAGE [1]

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TWO FIGURES

(Received for publication October 9, 1933)

The vitamin C potency of apples has been found to vary with the variety and with the length and mode of storage. It is of scientific interest, therefore, as well as of dietetic importance, to determine the vitamin C content of the different apple varieties and to ascertain the effect of storage upon the antiscorbutic properties of each important variety.

Experiments reported to date as summarized in table 1 indicate a wide range in the amount of apple required to prevent scurvy. The richest variety yet reported (Bracewell et al., '30, '31) is an English cooking apple, Bramley's Seedling, which protected guinea pigs from scurvy when fed in amounts as low as 3 gm. daily. For other English apples the minimum protective dose ranged from 10 gm. in three varieties to more than 20 gm. in three other varieties for which larger amounts were not tested. One variety furnished protection in 20 but not in 10-gm. amounts daily. The effect of storage was found by the English workers to differ, depending both upon the variety of apple and upon the temperature and composition of the atmosphere. Apples brought to England from other parts of the British Empire showed in general a lower vitamin C content than native apples of the same variety. This

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difference appeared ascribable to the necessary storage during transportation. Experiments with one variety, Bramley's Seedling, indicated no influence upon vitamin C value as a result of time of picking, character of soil, age of tree, or season.

TABLE 1

The antiscorbutic potency of apples

VARIETY	LOCATION	MINIMUM PROTECTIVE DOSE, GM.
Baldwin	Massachusetts[1]	4
Bramley's seedling	England[2]	3
Cox's orange pippin	England[2]	20, or slightly more
Dabinett	England[2]	10
Delicious	Massachusetts[3]	17 to 20
Delicious	Washington[4]	20 to 25
Golden delicious	Massachusetts[3]	12 to 15
Jonathan	Missouri[5]	20 to 25
King David	Massachusetts[3]	13
King Edward	England[2]	20, or slightly more
Lane's Prince Albert	England[2]	10
McIntosh	Massachusetts[3]	More than 20
Newton wonder	England[2]	10
Northern spy	Massachusetts[3]	4 to 5
Spitzenberg	Massachusetts[3]	10 to 11
Winesap	Massachusetts[3]	10
Winesap	Washington[6]	10
Woodbine	England[2]	20
Worcester pearmain	England[2]	20

[1] Fellers et al., '33.
[2] Bracewell et al., '30, '31.
[3] Fellers (personal communication).
[4] Present report.
[5] Hessler and Anderson, '30.
[6] Potter, '33.

In the United States, Hessler and Craig ('29) found that 20 gm. of Jonathan apples did not protect guinea pigs from scurvy. Hessler and Anderson ('30) subsequently reported that 25 gm. of this variety provided complete protection from September to November, while 40 gm. of stored Jonathans were required from December to May. Fellers ('33) found that more than 20 gm. of McIntosh and 17 to 20

gm. of Delicious were necessary for protection from scurvy. About 13 gm. of King David, 12 to 15 gm. of Golden Delicious, and 10 to 11 gm. of Spitzenberg appeared necessary for protection. Baldwins had a very high vitamin C content, 4 gm. being the minimum protective dose (Fellers et al., '33). The Winesap was found by Potter ('33) to be relatively rich in vitamin C, the minimum protective dose being 10 gm. daily. Potter and Overholser ('33) reported that apples from trees which had received a complete fertilizer (N.P.K.) for 4 years were somewhat richer in vitamin C than were apples from non-fertilized trees of the same district.

The investigation reported herein was conducted with Delicious apples grown in the State of Washington. The vitamin C value was determined in the fall, in the winter, and in the spring. The winter and spring experiments included apples that had been held at two temperatures: about 45°F. ('common storage'), in a fan-ventilated cellar having a relative humidity of 78 per cent to 82 per cent; and 32°F. ('cold storage') in a commercial cold storage plant. The maximum range of temperature in 'common storage' was 38°F. to 48°F.; in cold storage the extreme range was from 30°F. to 34°F.

METHOD

The vitamin C determinations were made according to the method described by Sherman and Smith ('31) except for a few changes here indicated. The duration of the test was in most cases 56 days. The basal diet was modified to contain cod liver oil and yeast because experience in this laboratory as well as that of Eddy ('29) indicates that more uniform results are obtained with a diet so modified. To supplement the iodized salt used and to insure an adequate amount of iodine, distilled water containing about 2.3 parts per million of iodine, added as potassium iodide, was given twice weekly. The amount of apple fed was not adjusted to the weight of each guinea pig as suggested by Sherman. Except in the winter tests, the weights of the animals were approximately 300 gm. in all cases. The animals were weighed every 2 days.

Weighed amounts of the basal diet were given every day and the net intake determined every 4 days.

MATERIALS

Through the courtesy of Dr. E. L. Overholser, of the Division of Horticulture of the Agricultural Experiment Station of the State College of Washington, Delicious apples, extra fancy grade, were obtained from two orchards, one at Chelan Falls and one near Monitor, Washington. No fertilizers had been applied to these trees for 3 years and 2 years respectively. The apples had been washed before packing to remove arsenic and lead residues to below the tolerance of 0.01 grains As_2O_3 and below 0.025 grains of Pb per pound of fruit.

Three sets of determinations were made: a) in the fall from October to December, b) in the winter from January to March, and c) in the spring from March to May. The fall determinations were made on apples held at 32° F. since presumably there would be less destruction of vitamin C at this temperature and the results would, therefore, represent freshly picked apples as nearly as possible. About 11 weeks elapsed between the picking and the final feeding of these apples to the guinea pigs. The winter and spring tests included apples that had been stored at 45°F. as well as those held at 32°F. The apples were washed, wiped, quartered, and cored. Any bruised tissue was removed. Radial sections were fed in which the natural proportions of skin and flesh were retained. Feedings were made 6 days per week.

EXPERIMENTAL RESULTS

The results obtained are summarized in figures 1 and 2. 'Positive controls' on orange juice and on cabbage are also shown.

Prevention of scurvy. The severity of scurvy, as indicated by the scurvy score, varied inversely with the amount of apple fed except that in the fall the guinea pigs receiving 5 gm. of apple showed a slightly higher scurvy score than their negative controls, probably because they lived about 10 days

longer so that the symptoms had more time in which to develop. Of the six receiving 10 gm. of apple, two died and all showed severe scurvy symptoms and a high scurvy score.

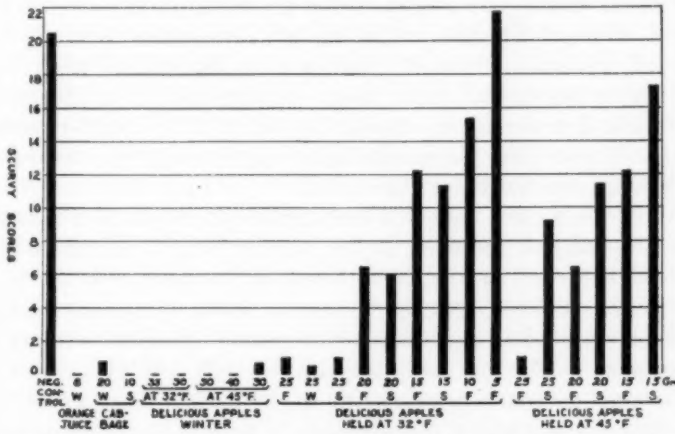

Fig. 1 Average scurvy scores of guinea pigs fed a vitamin C-free diet plus supplementary foods as indicated at foot of each column. F = fall, W = winter, S = spring.

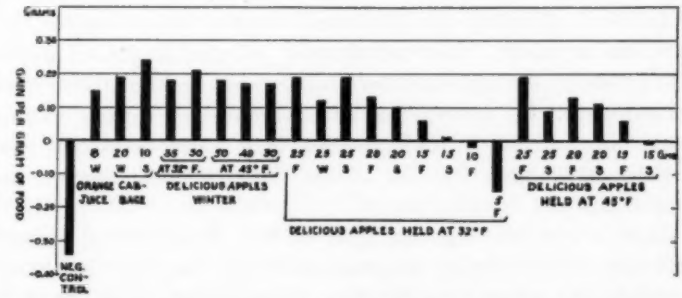

Fig. 2 Average gains per gram of basal diet of guinea pigs fed a vitamin C-free diet plus supplementary foods as indicated at foot of each column. F = fall, W = winter, S = spring.

All the animals fed 15 gm. of apple lived but showed severe scurvy. Those fed 20 gm. showed mild scurvy. With 25 gm. complete protection was given at all seasons with apples

stored at 32°F. Experience indicates that such apparent defects as contributed to the very low scurvy score of 1 in the spring and fall are probably of chance origin. No evidence of scurvy was found in the guinea pigs fed 25 gm. of 'cold storage' apples in the winter. A comparison of the results at three levels of feeding (15, 20, and 25 gm.) for the three periods of time shows no diminution in the antiscorbutic potency of the apples stored at 32°F. for 6 months and indicates that the minimum protective dose lies between 20 and 25 gm. fed 6 days per week.

With the guinea pigs fed the apples stored at 45°F., however, a marked increase in the spring scurvy score is observed for each of the three levels of feeding (15, 20, and 25 gm.). Twenty gm. of 'common storage' apples in the spring resulted in a scurvy score nearly identical with that produced by 15 gm. of apple in the fall. While a direct numerical comparison cannot be made at the other two levels of feeding, a similar relationship is apparent. This represents an increase of one-third in the amount of apple required to provide a similar protection from scurvy which, conversely, may be taken to indicate a loss of about one-fourth of the vitamin C potency of Delicious apples when stored for 6 months at 45°F. The fact that 50, 40, and 30 gm. of apples held at 45°F. gave protection from scurvy in the winter tests indicates that the destruction after 3 months' storage was not more than one-sixth, and possibly less, since no direct comparison was made for the 25-gm. level at that season and temperature.

Gain in weight. The net gain or loss in weight of animals suffering from varying degrees of scurvy has not, in the experience of some investigators (Richardson and Mayfield, '32), shown a direct relationship to the scurvy score. While considerable variability is apparent, particularly in averages of small numbers of animals, the average daily gains of the guinea pigs in these tests (where equal numbers of each sex were compared as far as possible) decreased as the scurvy score increased. It appears, therefore, that in these experi-

ments lack of vitamin C could be correlated directly with the growth failures observed. It is possible, of course, that the vitamin C-free diet lacked some unrecognized growth factor as well as the antiscorbutic factor and that both are present in Delicious apples in approximately equivalent proportions. A study of the efficiency with which the basal diet was utilized for growth seems to indicate, however, that lack of the antiscorbutic factor interfered with the absorption or metabolism of materials essential for growth. The average gains per gram of food ingested per day were similar for all guinea pigs receiving a protective dose of apple or other source of vitamin C but decreased as the allowance fell below the protective level. Apparently, therefore, vitamin C may be held responsible, indirectly at least, for the failure of the scorbutic animals in these experiments to make the gains in weight to be expected from the amount of food they ingested.

GENERAL DISCUSSION

These results indicate that Delicious apples contain about 0.04 to 0.05 unit of vitamin C per gram, or 1.1 to 1.4 units per ounce, according to the standard suggested by Sherman. This represents a relatively low value as compared with some other varieties tested. The outstanding place of Delicious, from the point of view of production in the United States (there were more Delicious trees than of any other variety in 1928 (Hampson, '33)), makes it of more than theoretical interest to consider the dietetic value of this fruit, particularly its value in vitamin C. The Delicious is generally eaten raw, a point worth considering, since, with few exceptions, cooking destroys or drastically reduces the vitamin C content of apples (Bracewell et al., '30; Fellers et al., '33; Kohman et al., '24; Hessler and Williams, '29). Until some standards can be set up which can safely be relied upon to preserve the vitamin C in cooked apples the use of the raw fruit should be encouraged in those households where, for reasons of economy as well as of taste, apples are depended upon to furnish a considerable part of the family's vitamin C require-

ment. Except for infants and young children, who should receive their vitamin C in some such concentrated and readily digested form as tomato juice or orange juice, raw apples might well be used more extensively, especially where they represent a relatively cheap source of vitamin C. The varieties of apple richest in vitamin C would obviously contribute, weight for weight, the largest amount of vitamin C to the diet. But a very large-sized apple like the Delicious, which averaged 200 gm. per apple in these tests, and which is nearly always eaten raw, may contribute an amount of vitamin C to the diet equivalent to that provided by an apple from one of the smaller though richer varieties, particularly those that are frequently cooked before serving. Since the peel contains a greater concentration of vitamin C than the flesh of the apple (Bracewell, '31; Fellers et al., '32), unpared apples provide more vitamin C than those from which the skin has been removed.

Since cold storage preserved the vitamin C content of Delicious apples successfully while 'common storage' permitted some destruction, it appears desirable to use cold storage whenever practicable and to maintain a temperature as near 32°F. as possible, when other types of storage must be used. Incidentally, the apples held at 32°F. retained a better texture and flavor and suffered less from overripeness and other forms of spoilage than those held at 45°F.

SUMMARY AND CONCLUSIONS

Delicious apples grown in Washington were found to have a vitamin C content of 0.04 to 0.05 unit per gram, or 1.1 to 1.4 units per ounce. No loss of vitamin C was evident after 6 months' storage at 32°F. A loss of one-sixth or less occurred during the first 3 months of storage at 45°F., and a loss of about one-fourth during storage for 6 months at this temperature.

Both the growth of the guinea pigs and the efficiency with which they utilized their food decreased as the vitamin C allowance fell below the minimum for protection from scurvy.

It is concluded that Delicious apples, although relatively low in vitamin C as compared to some other apple varieties, may make an important contribution of this vitamin to the diet because they are relatively large in size and are usually eaten raw and unpeeled. It is also concluded that the anti-scorbutic factor may have an indirect effect on growth due to failure of the scorbutic animal efficiently to utilize the essential nutrients in the food which it eats.

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STUDIES IN THE CONTROL OF DENTAL CARIES. II [1]

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IN COOPERATION WITH MARY CROWLEY, PHILIP JAY, DOROTHY G. HARD AND KATHRYN HENSEY
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ONE FIGURE

(Received for publication October 6, 1933)

Most people who are studying the cause and control of dental caries today believe that a well-balanced diet is a safeguard against the progress of this disease. Various nutritive constituents of the diet are regarded as specific protective agents. Metabolic processes related to calcium or phosphorus utilization or to acid-base balance are thought by many to exert a controlling influence. It is believed that this influence operates through causing changes either in tooth structure or in salivary composition, or by affecting the ability of aciduric organisms to grow in the mouth.

Many of the studies with children that have been reported cover rather short periods of time. Findings on first examination of teeth have had to be taken as describing the degree of activity of caries at the time of beginning the experiment. In some instances conclusions have been based on combinations of clinical and chemical evidence; in others, on bacteriological evidence of a qualitative nature considered in connection with clinical findings. In many instances conclusions have been based on clinical results obtained 6 or 8 months after some change had been made in the diet for one

[1] This work is a part of a group study of the cause of dental caries, being conducted under the auspices of the Children's Fund of Michigan, at the School of Dentistry, University of Michigan.

group of children, in contrast with similar evidence in another group in which the diet had not been changed. Sometimes changes in diet have involved a single dietary factor. In other instances, as in the daily addition of 1 pint of orange juice to the routine diet, many nutritive changes have been introduced.

In our studies we were fortunate in being able to conduct some investigations on children living in an institution. Yearly dental examinations over a period of years had revealed a surprisingly low incidence of dental caries among the children, without any conscious effort on the part of the managers of the orphanage to produce this result. Many of the children have lived in this institution for long periods of time. The same persons have been in charge for 6 or 7 years, during all of which time the same general policies have been followed in meal planning and food service. Due to the interest and cooperation on the part of those in charge, we had an unusual opportunity to study these policies. We hoped that we would be able to find out what dietary or nutritive principles were responsible for the unusual clinical results. At the same time we were able to make bacteriological and some chemical studies on the saliva of a selected group of the children. Distance from the laboratory made it impossible to make more extensive studies in these fields.

The cottage system is used in housing the children. The cottages are attractive and very well kept up. An unusually well-equipped recreation building and play field provide excellent recreational facilities. A large out-door swimming pool is in daily use whenever the weather permits. The children are a well-trained happy group, free from worry and from conditions leading to high nervous tension. The general atmosphere in the institution is that of a well-managed home. Bed time and mid-day rest periods are routine and adjusted to the age and physical condition of the children. Work and play are judiciously intermingled. The younger children attend school on the grounds, while those of junior and senior high school age attend school in the near-by town. The children are not permitted to go away from the institution

except during the summer months. Many of them have no homes to which to go.

Their general health has been very good. There is a hospital on the grounds with two nurses in constant attendance. With such facilities infectious diseases do not have as much chance of spreading as they do among children living in private homes and attending public schools. A pediatrician and a dentist from the near-by city are responsible for the necessary medical and dental care.

METHODS

The children are weighed and measured in May and November each year. These records were available to us as were their medical records. Inasmuch as the same policies in planning meals had been followed for several years previous to November 1932, the records at the end of May 1932 were used as the basis for the data presented in figure 1. This chart shows graphically the proportion of boys and girls of different age groups that varied from the generally accepted normal range of height and weight. The extent of individual variation is also indicated. The younger children had lived under this institution regime for an average of 2½ to 3 years, the older ones for an average of 5 or 6 years or more. Woodbury tables were used in calculation of so-called normal weights for age and height and of normal heights for age.

After the children are weighed in November, it has been customary each year, to give one teaspoonful of cod liver oil once a day to those children who are conspicuously under weight for their age and height. This dosage is continued through the winter months only. No special attention has been given, however, to those markedly under average height for their age.

Dental examinations. In October 1928, our group first began to make yearly examinations of the teeth of the children living in this institution. Those who were over 6 years old at that time were selected. It has not been possible to have

bite-wing x-rays taken, but individual charts have been kept. Successive findings from year to year have been recorded on these charts with inks of different colors. Each succeeding year until October 1931, examinations were limited to this same group of children. By October 1931, this original group had been considerably reduced in numbers, due to withdrawals

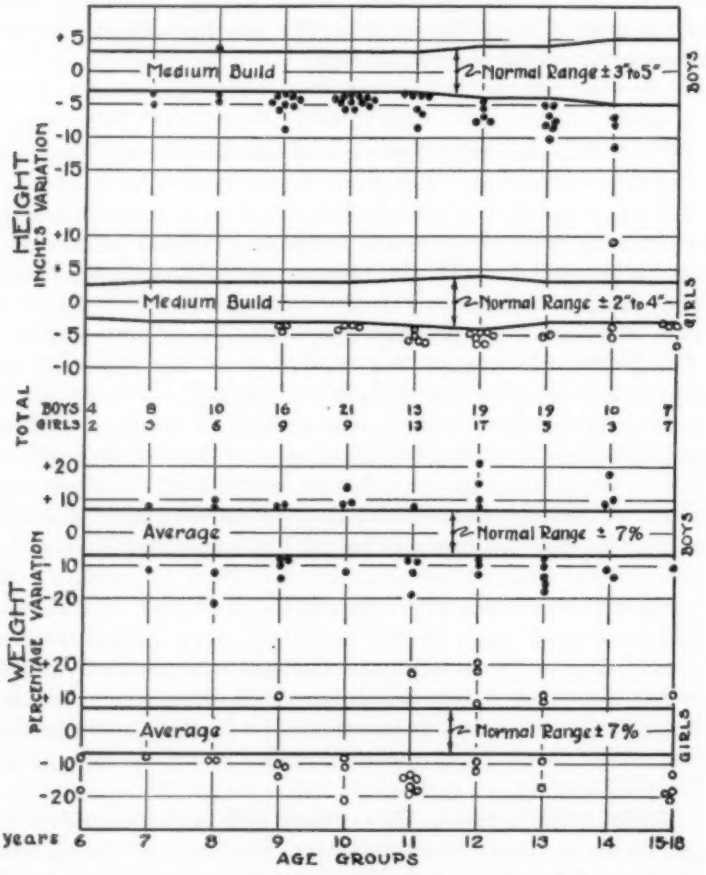

Fig. 1 Abnormalities in height and weight in a group of 127 boys and 74 girls who had lived in an orphanage for an average of 4 years.

from the institution. All other children over 6 years old were then added to the list. From October 1928 to October 1931 only very urgent dental conditions in the children who were under our observation were cared for by the local dentist. Most cavities were left open.

The last examination was made in June 1933 instead of October, because of the probable necessity of our researches having to be discontinued. At the end of June 1933 we were in possession of yearly dental records for 49 children covering 4½ years, for 109 others 1½ years and for 11 covering 1 year. At this time all individual records were carefully studied and a yearly diagnosis made for each child describing the progress of dental caries during each year. The following symbols were used:

C— indicated that no new cavities had developed. If used for the description of the result of the first examination, it meant that the child had had no cavities at that time.

C± was used to represent a very slight amount of activity.

C+ signified a fair amount of activity.

C++ represented considerable activity.

C+++ indicated extreme susceptibility.

In each case the symbol represented change since the last previous examination. In table 2 will be found a summary of the progress of caries in these children, classified according to the periods of time indicated.

Diet. During the year 1931 our efforts were directed to general observations on the food the children were receiving and to securing full information from the person in charge, concerning the general policies that had guided her in planning their menus. We have copies of the menus for several years back. Comparison of these with the statements made gave a close check on the accuracy of her description of these policies.

Children past 8 years old received milk and oleo routinely for breakfast. Occasionally these foods were served for supper. Butter was not available. A high proportion of bread and other cereal foods was used regularly. Many

vegetables and some fruits are raised each year on the institution farm. Apples, carrots, tomatoes, onions, potatoes, cabbage, beets and turnips were abundant at all times. Celery, spinach, peas, string beans, corn, berries, etc., were given to the children in season. Carrots, onions, cabbage, turnips and tomatoes were frequently served raw. Oranges were given to the older children only during the Christmas holidays, except as sent in to individual children at other times by relatives. Canned berries, peaches and apples, together with dried prunes and peaches were purchased for their menus; fresh bananas were purchased occasionally. Eggs were available to the chlidren in the spring, during which period they were produced on the farm in considerable quantity. The only desserts that were given regularly to the children were fruit gelatin[2] for Sunday dinner and cookies that were served to them once a week. Raw apple was used in place of dessert for dinner and supper at practically all other times. Fresh and smoked meats were purchased for the children's menus. Meat was served almost every day, but portions were quite small. Candy that was sent in to the children was not given to them. Parents were urged to limit food gifts to fresh fruits.

There were three features about this general type of diet that seemed to us might bear a relation to the low incidence of caries among the children. One was the habitual use of hard fibrous raw fruit at the close of meals. Another was the fact that foods containing added sugar were rarely used. Lastly, the food was always of uniform quality and was eaten at regular times.

Because all of the children ate in the same dining room, it was thought unwise to attempt to arrange for groups of children to receive different dietary supplements of interest to us in our research program. A group of 60 girls, all living in the same cottage and all over 6 years of age, was selected, however, early in January 1932. We wished to observe whether or not the order in which raw fruit and vegetable

[2] Jello.

was eaten at meal time made any difference in dental or bacteriological findings. For 1 year these children ate their raw fruit at the beginning of meals instead of the close as had been the usual custom. All other children continued their customary practice in this respect. Twenty girls of different ages from this special group and an equal number of similar ages from among the rest of the children were selected as subjects for making bacteriological studies on the saliva.

Eight each from these 2 groups of 20 were selected to study the food intake of representative children in the dining room. Three such dietary studies were made. Because of the general uniformity in types of meals served, we were certain that the children received the same kind of food while our studies were in progress as they would have received otherwise. On our part we followed the same policies in food service to the diet group that were followed throughout the dining room. Before each meal we obtained information from attendants in the dining room as to whether second helpings were to be given, size of first helpings, etc. Food was prepared separately for the 16 whose food intake was being studied, but proportions were kept similar to those used in preparing the larger quantities. The cooks weighed all constituents of various foods served to the diet group and weighed the final cooked product for us. All food consumed by these 16 girls during the time dietary studies were in progress was weighed.

The 3 studies covered the following periods: 3 days at the end of May 1932, 4 days in September 1932 and 7 days in March 1933. As far as possible the same girls were studied each time. Because of a few withdrawals, however, there had to be occasional substitutions. New children were always selected from the 2 groups of 20 to which we have already referred. The results of May and September 1932 studies were combined, making a full week for comparison with the week studied in 1933. As a basis for our calculations we used the compilation of generally accepted data on food composition that has been published by the Department of Dietetics, University Hospital, Ann Arbor (Waller, '32). Table 1 gives

a summary of these results together with a summary of similar data covering 54 days, for 9 girls who were in our special diet group at University Hospital (Koehne et al., '34; Koehne and Morrell, '34).

In November 1932, after our dietary studies for the two periods in 1932 had been completed, we secured the cooperation of the physician and of those in charge at the institution in making a few simple changes in policy. These changes resulted in the children thereafter receiving regularly more milk and egg, some butter and a greater proportion of whole cereal products than of the milled varieties. The hot dishes served for dinner and supper were higher in nutritive value and stewed fruit was included in the breakfast menu. Further changes were not deemed wise in view of the stress of general financial conditions. Table 1 shows a direct comparison of food intake, as measured in March 1933, 4 months after these changes were instituted, with the results previously obtained.

Bacteriological studies. From December 1931 through April 1933 quantitative estimations of the concentration of Bacillus acidophilus in saliva were made once a month for the selected group of 40 children previously described. The method developed by Hadley ('33) was used in this work. No tests were made during the summer months. Thirteen such determinations were made for 25 of the original group. There were 15 withdrawals during the first year. Qualitative tests for the presence or absence of aciduric organisms in the saliva were made at each time that quantitative determinations were made. The method used was that which has been described by Jay and Voorhees ('27) and by Bunting, Nickerson and Hard ('26).

Chemical studies. During the latter part of May 1932, and in March 1933, while studies of the food intake of 16 girls were in progress, the carbon dioxide capacity of the saliva of the same girls was measured. The macro method used was the one developed by Van Slyke and Cullen ('17) for similar determinations on blood serum. One cubic centimeter of saliva was used for each determination. The saliva was col-

TABLE 1

Comparative study of the food intake of sixteen girls living in an orphanage and of nine girls at University Hospital

	ORPHANAGE		HOSPITAL
	1 week 1932	1 week 1933	First 6 to 11 weeks in hospital
Average age (years)	10.9	12.0	8.87
Years spent in institution	3.7	4.3	
Average height (inches)	52.5	55.37	52.37
Deviation from normal height/age	— 3.2	— 2.54	+ 0.8
Normal range of deviation	+ 3.2	+ 3.4	+ 3.0
Average weight (kilograms)	29.6	35.3	30.8
Deviation from normal weight/height/age	— 4.0%	— 0.3%	+ 3.0%
Normal range of deviation	± 7.0%	± 7.0%	± 7.0%
Protein: average daily grams	61.8	75.8	74.0
Grams per kilogram	2.1	2.25	2.4
Per cent from animal sources	40.7	43.1	65.75
Fat: average daily grams	59.6	74.8	99.0
CHO: average daily grams	249.0	299.0	233.0
Added sugar: average daily grams	15.0	15.3	29.2
Calories: average daily	1759	2169	2074
Calories per kilogram	60.8	64.2	66.4
Calories per inch height	33.8	39.3	39.5
Per cent from protein	13.9	14.0	14.2
Per cent from fat	30.3	31.0	42.8
Per cent from CHO	56.0	55.1	43.0
Per cent from added sugar	3.4	2.8	5.6
Calcium: average daily grams	0.64	0.99	1.39
Phosphorus: average daily grams	1.11	1.52	1.54
Iron: average daily milligrams	12.0	13.7	13.9
Acid value: cubic centimeter N/1 acid	20.9	23.4	19.0
Base value	32.8	43.8	49.4
Excess base value:	11.9	20.4	30.4
Average amounts of different kinds of food eaten daily (grams)			
White bread	131	84	0
Whole wheat bread	54	91	71
Other cereal foods	261	152	96
Cooked dry legumes	51	61	0
Totals	497	388	167
Milk, all purposes	397	632	985
Potatoes	93	96	141
Raw vegetables, fruit, tomato juice	197	153	189
Cooked vegetables and fruit	138	226	251
Totals	428	475	581
Butter	0	12	29.5
Oleo	11	7	0
Miscellaneous: fat-rich foods	19	13	33
Cane sugar	2	2	4
Miscellaneous sweets	12	7	10
Eggs	8	21	38
Meat, fish, poultry	49	21	66
Nuts, peanut butter	8	12	2
Cheese	0	16	9
Totals	65	70	115
Percentage distribution of calories among different classes of foods			
	[1]		
Cereals, bread, legumes	44.3	39.6	15.8
Milk	16.3	20.3	31.8
Vegetables and fruit	12.9	14.9	17.1
Fat-rich foods	10.1	13.3	18.1
Sweets	4.7	3.2	5.7
Meat, egg, cheese, nuts	11.7	11.3	11.4

[1] Daily caloric intake markedly low during this period. Results do not have the significance they should have, if caloric intake had ben adequate as in other two studies.

lected under oil, through a long-stemmed glass funnel. Saliva flow was always stimulated by the slow chewing of a small piece of paraffin.

DISCUSSION

Diet in relation to the height and weight of the children. The results of the dietary studies made during 1932 are reported in table 1. As evidence that this summary for 16 children is representative of the food intake of the children who lived in the institution during the preceding 6 or 7 years, we submit the data found in figure 1. Only 1 boy and 1 girl were taller than the average tall child of their ages. Forty-nine of the 127 boys represented were shorter for their age than the average boy of short stature. The same was true of 27 of the 74 girls. As will be seen by reference to figure 1 there was a massing of this condition from 9 years of age on for both sexes.

Weight data presented in figure 1 should be interpreted in comparison with the height record. Estimation of the so-called normal weight was made, as is customary, according to the age and actual height of each child, not according to his so-called normal height for age. Twenty-two of the boys were more than 7 per cent below normal average weight for their age and height, while 16 were more than 7 per cent above this average value. In the case of the girls 26 were below this normal range in weight for their age and height and 8 were above this average range. Figure 1 indicates that the distribution between over and under weight was more uniform in the boys of the different age groups than among the girls. In most age groups a higher percentage of the girls were under than were above the average weight range for their height and age. Within a period of 6 months following November 1932 when the changes were made in diet, there was a doubling of the gain in weight and a 15 to 20 per cent increased gain in height in a group of 85 boys and 54 girls, in comparison with gains made by those same children in a similar 6-month period the year before.

Relation of dental findings to height-weight status, age and sex. A study was made of the possible correlation between the height or weight status of the children and their degree of susceptibility to caries. About the same proportion of children who were stunted in height and were more than 7 per cent above or below average in weight for their height and age, was found in each of the dental classifications.

There are 109 boys and 60 girls represented in table 2. The group in whom caries remained inactive for the periods of time under observation included 55 boys and 28 girls. Fifty-

TABLE 2

Summary of dental findings for 169 children who were under observation for varying periods of time

PERIOD UNDER OBSERVATION	4½ YEARS		1½ YEARS		1 YEAR	
NUMBER OF CHILDREN	33 boys	16 girls	68 boys	41 girls	9 boys	2 girls
	Number	Per cent	Number	Per cent	Number	Per cent
Immunes	6	12.2	26	23.8		
Inactive since first examination	17	34.7	52	47.7	9	81.8
S1 to moderate activity for first 1 to 2 years, inactive for last 2½ to 3½ years	5	10.2				
S1 activity throughout period	9	18.2	20	18.3		
Moderate activity throughout period	8	16.3	8	7.3	2	18.2
Considerable activity throughout period	4	8.1	3	2.7		

five per cent of these boys were under 10 years of age in contrast with 40 per cent of the girls. Eighteen boys and 7 girls exhibited moderate to very active caries while under observation, 7 of the boys and 4 of the girls being under 10 years of age. Two-thirds of the 19 boys and 13 girls classed as immunes were under 10 years old. It seems significant that much the larger proportion of so-called immune children was found in the younger age ranges. Likewise almost twice as high a proportion was found among those under observation for just 1½ years as was found among the 49 who were observed for 4½ years. Time seems to lower considerably the

proportion of individuals who can be called immunes. We hope to continue our observations on these children in order to determine how many of the younger ones remain immune over a period of years.

Relation between dental and bacteriological findings. Ten to 13 tests were made on the salivas of each of 25 girls between December 1931 and April 1933, to determine the concentration of Bacillus acidophilus in the saliva. Twelve of these girls belonged to the special group who, throughout the year 1932, ate their raw fruit and vegetable at the beginning of meals. The remaining 13 were of similar ages but had always eaten their raw fruit and vegetable at or near the close of meals. None of the 25 girls belonged to the group of immunes, for all had had cavities on first examinations.

In table 3 will be found the dental diagnoses and the bacteriological findings, qualitative and quantitative, for 9 representative children in the group of 25. Wherever the quantitative plate is recorded as negative but the qualitative test as positive, it may be assumed that there were probably less than 100 aciduric organisms present per cubic centimeter of saliva. For purposes of routine interpretation in this laboratory, it has been customary to consider counts less than 5000 per cubic centimeter as low, those between 5000 and 50,000 as moderate, and those over 50,000 as high. The continuous presence of these organisms in the saliva over long periods of time is typical of caries activity. In actively susceptible cases, the counts usually vary from moderate to high, although some may be low.

Two additional children had records resembling that of V.S. Such records are typical of caries inactivity, yet the November 1932 dental examination for V.S. and D.B. showed slight activity as did the June 1933 examination for A.R.P. These 3 cases and 1 other (R.B.), who had a bacteriological record resembling that of J.G. and a dental record similar to that of V.S., were exceptional cases in that their records failed to show a direct correlation between the dental and the bacteriological findings.

The salivas of 3 children were consistently negative to B. acidophilus, both qualitatively and quantitatively, and none developed cavities during the year and a half of observation. One other child had a record resembling that of A.K., while another child's record was similar to that of L.P. Eight other children had records like that of J.G.

TABLE 3

Dental and bacteriological findings for nine representative children

DATE		V.S.	J.G.	A.K.	L.P.	W.M.	B.STA.	V.U.	B.STE.	R.S.
Oct. 1931	Diagnosis	C+	C+	C+	C+	C++	C+	C+	C+	C++
12–18–31	Quant. test	0	0	0	0	0	0	36,075	0	4,475
	Qual. test	neg.	neg.	neg.	neg.	neg.	neg.	pos.	pos.	pos.
1– 4–32	Quant. test	0	0	400	370,000	0	0	75,000	37,312	8,375
	Qual. test	neg.	pos.	pos.	pos.	neg.	neg.	pos.	pos.	pos.
1–21–32	Quant. test		0		0	0	0	9,075	1,200	0
	Qual. test		neg.		pos.	neg.	neg.	pos.	pos.	pos.
3– 3–32	Quant. test	0	0		0	0	0	10,775	8,075	43,500
	Qual. test	pos.	neg.		neg.	neg.	neg.	pos.	pos.	pos.
4– 5–32	Quant. test	0	0	0	0	0	0	8,700	216,000	0
	Qual. test	neg.	neg.	neg.	neg.	neg.	neg.	pos.	pos.	pos.
5– 9–32	Quant. test	0	0	0	0		0	0	27,000	
	Qual. test	neg.	neg.	neg.	pos.		neg.	neg.	pos.	pos.
6– 6–32	Quant. test	0	0	0	0		0	0	288,000	0
	Qual. test	neg.	neg.	pos.	neg.		neg.	neg.	pos.	pos.
9–20–32	Quant. test	0	0	0	0	0	1680	0	9,000	0
	Qual. test	neg.	neg.	neg.	neg.	pos.	pos.	neg.	pos.	pos.
11– 1–32	Diagnosis	C±	C—	C—	C—	C—	C—	C—	C—	C+
	Quant. test	0	0	0	0	0	0	0	12,000	0
	Qual. test	neg.	neg.	neg.	neg.	neg.	pos.	neg.	pos.	neg.
12–17–32	Quant. test	0	0	0	0	0	302	0	9,075	0
	Qual. test	neg.	neg.	neg.	neg.	neg.	pos.	neg.	pos.	neg.
1–28–33	Quant. test	0	0	0	0	11,700		0	100	0
	Qual. test	neg.	neg.	neg.	neg.	pos.	pos.	neg.	pos.	neg.
3–20–33	Quant. test	0		0	0	121,600	125	0	8,200	15,062
	Qual. test	neg.	neg.	neg.	pos.	pos.	pos.	neg.	pos.	pos.
4–16–33	Quant. test	0	0	0	0	22,800	0	0	8,600	11,900
	Qual. test	neg.	neg.	neg.	neg.	pos.	pos.	neg.	pos.	pos.
June 1933	Diagnosis	C—	C—	C—	C—	C±	C±	C—	C+	C+

Quant. test gives the number of B. acidophilus per cubic centimeter of saliva.

The records of W.M. and B.Sta. are typical of caries inactivity changing to activity. The opposite condition is seen in the findings reported for V.U. B.Ste and R.S. have records that are typical of caries activity. In these last two cases we see caries activity associated with rather low counts in R.S. as well as with moderate to high counts (B.Ste.). Undoubtedly variations exist in individuals in rate of development of cavities. Then, too, improvement in methods of collecting saliva for quantitative counts may later lead to results that are more comparable from person to person than are present methods.

Out of the 25 cases discussed, all but 4 show a direct correlation between dental and bacteriological findings, making a correlation of 84 per cent. This compares favorably with the 88.9 per cent correlation found in our hospital studies (Koehne, Bunting and Morrell, '34).

During the year 1932, the incidence of new caries was practically the same among all the children as during the previous years, in spite of the fact that all existing cavities had been filled and all of the teeth cleaned at the beginning of the year. This had not been done in previous years.

Relation between dental findings, diet and carbon dioxide capacity of saliva. The results of the dietary study made in March 1933, 4 months after certain policies in meal planning had been changed, showed approximately a 10 per cent increase in intake of available acid. The excess base intake was about 70 per cent higher than on the type of food consumed previous to November 1932.

Determinations of the carbon dioxide capacity of the saliva of the 16 children in the diet groups of May 1932 and of March 1933 gave the following results:

In May 1932, the average for the group was 20.35 cc. CO_2 per 100 cc. of saliva (range, 11.0 to 31.1). The average of the values obtained in March 1933 was 22.04 cc. CO_2 per 100 cc. of saliva, the values ranging from 10.8 to 30.4. The alkaline reserve of the saliva in March 1933 was only about 8 per cent higher than in May 1932, while the diet showed a 70 per cent increase in available base.

Twelve of the 16 girls in the group in March 1933 had been in the group in May 1932. Seven of these 12 girls had remained inactive to caries since October 1931. In May 1932 the average CO_2 capacity of their salivas was 20.9 cc. per 100 cc. of saliva (range 13.9 to 31.1), while in 1933 it was 22.0 cc. CO_2 per 100 cc. of saliva (range, 15.4 to 30.4).

Four of the 12 showed a slight degree of caries activity during the year and a half. In 1932 the average carbon dioxide carrying power of their saliva was 21.75 cc. CO_2 per 100 cc. of saliva (range, 13.5 to 25.0); in 1933 it was 23.6 (range, 17.6 to 28.5).

The one child who was definitely susceptible to caries had a CO_2 capacity for the saliva of 11.0 cc. per 100 cc. of saliva in 1932 and of 13.3 in 1933. These values would have more significance if there had been more children in this group giving correspondingly low values. As it stands the results for this 1 child have to be compared with values of 13.5 and of 17.6 cc. CO_2 per 100 cc. of saliva for 1 child and of 13.0 and of 15.4 cc. CO_2 per 100 cc. of saliva for another child, both belonging in the group that had remained inactive to caries.

As was found in the more detailed studies on a group of children in University Hospital (Koehne et al., '34) whose food intake was accurately measured over rather long periods of time, we were unable to find any significant quantitative correlation between the available base value of the diet and the alkaline reserve of the saliva as measured by its carbon dioxide carrying power. We also found no consistent relationship between the concentration of this component of the saliva and the progress of caries susceptibility. Such a possible relationship had been indicated by Hubbell ('33) in tests made on the saliva of a group of school children living in Ann Arbor.

Dental findings in relation to diet. Attention is again called to table 2 which presents a summary of the progress of caries among the children living in this institution. It will be regarded as unusual for 57.1 per cent of a group of children of

these ages to remain immune or inactive to the progress of this disease for the last 2½ to 4½ years that they were under observation. This proportion was increased to 71.5 per cent when observations were limited to 1½ years, and to 81.8 per cent for those observed for only 1 year. Only those children are listed as immunes who were free of caries on admission and who remained free of cavities during the time indicated. The usual amount of decay was found among all other children on first examination.

No higher incidence of caries was noted in the group of 60 girls during the year in which they ate their raw fruit and vegetable at the beginning of meals than had been found in the same children during previous or subsequent periods or than was observed among the other children who had continued their customary practice of eating such food at or near the close of meals. This observation was confirmed in carefully controlled studies made on some of the children in our diet group at University Hospital (Koehne et al., '34). Also, in these latter studies, we found that no special advantage was gained in controlling caries by eliminating the few simple desserts in our standard diet (table 1, right hand column) and substituting raw apple.

Between the October 1932 examination and that of June 1933, during which time the nutritive value of the diet showed considerable improvement, there was no apparent change in degree of susceptibility over that noted in previous years. With the exception of this final 6 months' period, the type of diet had, for several years, corresponded to that summarized in the left hand column of table 1. Therefore the low incidence of caries noted in table 2 had prevailed on a diet in which 40 to 45 per cent of the caloric intake was from starchy food, largely refined cereals.

Mellanby and Pattison ('32) have claimed that high cereal consumption encourages decay of the teeth by interfering with calcium and phosphorus utilization, through increasing the need for vitamin D. Hawkins ('31) has insisted that high cereal consumption favors the progress of caries by encourag-

ing film formation because of the gluten content of wheat. If either of these claims are justified, why has not caries been rampant in this institution? The children's mouths have remained unusually clean in appearance. Calcium and phosphorus intakes, except for the last 6 months, were consistently lower than usually advocated for growing children. The children play out of doors a great deal, but probably no more than the majority of children attending public schools and living at home. The small doses of cod liver oil given, for several months each winter, to children who were considerably under weight, could not account for the low incidence of caries in the whole group. We found approximately the same incidence of caries among the underweight children who had been given this dosage of cod liver oil for the past 1 or 2 winters, as indicated in table 2 for the whole group. We therefore do not feel justified in concluding that vitamin D was responsible for the clinical findings. Certainly the low incidence of caries cannot be explained on the basis of optimum intake of calcium and phosphorus.

We studied the incidence of caries separately among the children who had attended junior or senior high school for the past 1 or 2 years, compared with that found among those attending school on the grounds. Those attending junior high school return to the institution for lunch; those attending senior high school carry their lunch to school. Both of these groups of children assist in the children's dining room or in the other dining rooms on the grounds, employees', nursery, hospital, etc., and have some access to foods not available to the younger children. Again, however, the rate of incidence of caries was approximately the same, as among the whole group.

It has seemed to us that there are two possible dietary explanations for the clinical findings. One is the extremely low intake of artificially sweetened food. The other is the absolute regularity of meals and the uniformity in the quality of food served. Perhaps there are other explanations not evident to us at this time.

Drain and Boyd ('30), Hawkins ('31), Davis ('29) and others have publicly recommended the use of types of diets which have given satisfactory results in arresting the progress of caries in a high percentage of their cases. All have recommended well-balanced diets with restriction in the use of highly sweetened food. Hawkins has also insisted upon a low intake of cereal food. Davis has prescribed, in addition to the diet, the use of certain of his patent preparations. Each has explained the resultant beneficial effects according to his own theory. We can find no published evidence that diets, which include the unrestricted use of artificially sweetened food, have given successful results in stopping the progress of caries in large numbers of cases. Many groups of primitive people can be found who are free of caries. Their diets are usually limited to a few simple natural foods.

Results of experiments conducted by us on a group of children in University Hospital (Koehne et al., '34) living under strictly controlled conditions, have seemed to indicate that a well-balanced diet containing restricted amounts of sugar, is able to control the progress of caries in a rather high percentage of cases. Diets equally valuable nutritionally but containing a high percentage of calories from sweets, seemed to facilitate its progress. In these orphanage studies, however, we found that equally good results were obtained on a larger number of children observed for longer periods of time, when the children were on a type of diet much less satisfactory nutritionally but even lower in sugar content than that used by us in our hospital group. (Compare data given in table 1 for details of diets used by the two groups of children.)

Observations made by us on a group of children attending public schools in Ann Arbor and living in their own homes have seemed to indicate that irregularities in meals and in type of food eaten day by day, associated in most cases with the liberal use of artificially sweetened food, encouraged cavity formation.

If, during the past 6 or 7 years, the children in this institution had received a type of diet similar to that indicated in the right hand column of table 1, we are unable to say whether or not the incidence of caries would have been still further reduced. This type of diet corresponds to that recommended by Boyd and Drain and others. We are confident that the use of the better balanced diet would have eliminated most of the abnormalities in height and weight recorded in figure 1.

It will be noted on referring to table 1 that, on the regime preceding November 1932, the average daily caloric intake was below that deemed desirable for growing children of these ages. Suppose, as is done in many orphanages, that this institution had made a practice of accepting all gifts of confectionery which are so frequently made by well-meaning business firms, and had dispensed such food freely to the children. Undoubtedly there would not have been a deficiency in caloric intake. Since the children have excellent appetites, they propably would have continued to eat everything available in the dining room. The general diet would have been just the same, the caloric intake would have been adequate instead of deficient, but a much higher proportion of the calories would have been derived from added sugar. What would have been the effect of such a regime on caries incidence? We offer no prediction, but we know several similar institutions that follow this practice. The yearly incidence of caries among their children corresponds more to that found among public school children.

The secret of controlling caries activity through diet may, for the majority of persons, lie more in what is withheld from the diet than on the nutritional adequacy of what is permitted. The results of our observations in this institution would seem to point in this direction.

In conclusion we wish to call attention to one factor, the importance of which has been brought home to us many times in our recent studies and which may explain some of the discrepancies in published data. It is not safe, in dealing with human subjects, to base conclusions as to the effectiveness of

various experimental measures in arresting caries solely on short time observations of clinical results. The incidence of caries in the same group in a similar period preceding the experimental periods, should be reported for comparison. Cavities may remain unchanged for long periods of time.

Only 32 of the 169 children represented in table 2 were free of caries when examined in October 1931. The remaining 137 had teeth showing varying amounts of decay, open cavities in many instances. If we had begun to give viosterol or other specific treatment in October 1931, without any previous knowledge of caries incidence among the children, and had based our conclusions on clinical findings in October 1932, we would have been able to present striking evidence for the beneficial effect of our treatment, whatever it had been. Yet 22 of these 137 children had had no new cavity formation for 1 to 3 years previous to October 1931. During the following year, which was the first year of observation for all but 49 of the children, a total of 83 showed no progress of caries activity, even though no fundamental change had been made in the dietary or in other conditions.

SUMMARY AND RESULTS

1. The progress of caries is reported among 169 children living in an orphanage. They were under observation for periods varying from 1 to 4½ years.

2. Improvements made in the diet of the children brought about marked improvement in the height and weight of most of them and some increase in the alkaline reserve of the saliva. This latter increase was not at all proportional, however, to the increased alkaline value of the diet.

3. Bacteriological tests on the saliva of a selected group of these children gave results that checked with the dental findings in 84 per cent of the cases studied. The small percentage of cases in which the dental findings could not be satisfactorily explained on the basis of the quantitative bacteriological findings was slightly lower than observed in our studies of children in University Hospital.

4. No correlation could be found between the dental findings and any of the following factors:

a. Height or weight status of the children.

b. Sex.

c. The carbon dioxide carrying power of the saliva.

d. The intake of calcium or phosphorus or vitamin D.

e. Probable access to other food.

f. The order in which hard fibrous fruit was eaten.

5. The low incidence of caries among the children could not be explained on the basis of the superior nutritional value of their diet. Almost half of the caloric intake was from starchy food.

6. There were two other dietary factors that may explain why the incidence of caries was so low in this institution. One was the low intake of artificially sweetened food. The other was the great regularity of the meals and the uniformity in quality of the food served. If these were the controlling factors, we offer no explanation of how they operated. We merely submit our evidence in support of them.

Acknowledgment is made of the assistance rendered by Maxine Turner and Adelia Grieber, department of dietetics, University Hospital, for assistance in making some of the dietary studies. We wish to express to the superintendent and to his wife, who was in charge of food, our appreciation for their constant interest and careful cooperation in every detail of these studies.

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URINARY EXCRETION OF CITRIC ACID

I. EFFECT OF INGESTION OF LARGE AMOUNTS OF ORANGE JUICE AND GRAPE JUICE

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Increase in the alkalinity of the urine and in organic acid excretion resulting from the ingestion of fruits or fruit juices has been reported by Blatherwick and Long ('22), McLaughlin and Blunt ('23), Chaney and Blunt ('25), Saywell ('32), Saywell and Lane ('33), and Clouse ('33). In general this increase in organic acid excretion when fruit juices are fed has been interpreted to mean that the ability of the body to oxidize the particular organic acids represented has been overstepped, with the consequent elimination of the excess in the urine. There is some evidence, however, that the organic acids eliminated in the urine are not remnants of unoxidized fruit acids, but that they are of metabolic origin. In this connection, Clouse cites the increase in uric acid excretion obtained by McLaughlin and Blunt ('23) on a diet high in fruits. Clouse ('33) found that the ingestion of large amounts of orange juice and grape juice likewise increase uric acid excretion.

Recently the functioning of organic acids as a part of the acid-base balance regulatory mechanism of the body has received considerable attention. Increase in the total organic acid output of the urine on administration of alkali in the form of sodium bicarbonate was noted by Fanconi ('28) and by Fasold ('30), which suggests that organic acid radicals of

metabolic origin furnish a means of freeing the body of excess base. The increased alkalinity produced by fruits and fruit juices might then account for the increased organic acid excretion observed when these are fed in large amounts.

Of the organic acids concerned in acid base balance regulation citric acid has received the most attention and is the one which at present is being most extensively investigated. Its occurrence in the urine was first demonstrated by Amberg and McClure ('17) who considered a direct food origin very improbable. Fasold ('30) concluded from one of his studies that a considerable part of the citric acid excreted is of food origin, but, at the same time, he suggests a connection of citric acid with an acid-base balance regulatory mechanism. In another study ('30) he presents additional evidence for the latter view, which is further supported by the work of Östberg ('31).

It then appears that the assumption that the excreted organic acids can be taken as a measure of the unutilized ingested organic acids is open to question. It was felt that this subject might profitably be further investigated by following a particular organic acid contained in fruit juices, determining in every case both the amount of the acid ingested and the amount excreted. In none of the fruit juice studies reported has this been done. Citric acid was chosen as the particular acid of investigation because of the interest in it as a component of an important class of fruits and as a probable metabolic product functioning in acid-base balance regulation. Orange juice and grape juice were selected because of the readily available data from their use in other studies on urinary acidity. Their great difference in citric acid content also commended them for the purpose of this study.

EXPERIMENTAL

Two separate studies were made, using four subjects for each study. The subjects were young graduate women of the nutrition department of The University of Chicago. Each study was divided into two 8-day periods. During the first

period, the subjects received a basal diet only. In the second period the basal diet was supplemented with 1000 cc. orange juice daily for one study, and for the other study, two of the subjects received 1000 cc. orange juice and two of them 1000 cc. grape juice daily. The subjects were kept on the basal diet for 3 days before collections and analyses were begun, and, likewise, a 3-day preliminary or adjustment period was allowed when the orange juice and grape juice additions were made. The analyses included determination of pH, titratable acidity, organic acids as a whole and citric acid.

The basal diet in the first study, which we shall designate diet A, consisted of the following: Apple sauce, 100 gm.; canned tomatoes, 100 gm.; thin cream, 5 tbs.; lean beef, 90 gm.; eggs, 90 gm.; head lettuce, 50 gm.; Irish potatoes, 150 gm.; cornflakes, 20 gm.; whole milk, 1¼ cups; canned peaches, 100 gm.; coffee, 1 to 2 cups; bread, butter, vanilla wafers, and salad oil in amounts to complete the caloric requirements of the different subjects. Diet B, which constituted the basal diet of the second study, differed in the following respects from diet A. The meat was reduced to 75 gm.; thick cream, ½ cup, replaced the thin cream; the milk was omitted and shredded wheat biscuits (27 gm.) were used instead of cornflakes. Calculation of the potential acidity and alkalinity of the basal diets showed slight excess acidity (table 1). The addition of the fruit juices rendered the diets distinctly alkaline.

The caloric needs of all of the subjects while on the basal diet were evidently met, as was shown by the fact that weights remained constant, and in some instances the subjects showed small gains. The difference in caloric value between the basal diet and the basal diet with the fruit juice additions was, therefore, considered to be of no consequence in this study.

All food was weighed, prepared, and served in the laboratory, the subjects reporting for meals at 7:30, 12:30 and 6:30 each day. The orange juice was prepared from California oranges with an electric squeezer, so that it included a large part of the crushed pulp. The grape juice was a well-known commercial brand.

The collections of urine were made in clean wide mouth bottles with a sufficient layer of toluene to protect the urine from the air. At the end of the 24-hour period the specimens of each subject were thoroughly mixed and the volume recorded. The pH and titratable acidity were determined at once and organic acids within a few hours. The citric acid determination was begun within 24 hours, but the method involved steps requiring several days for completion.

TABLE 1

Acid base values of basal diets and fruit juice supplements (calculated from Sherman's figures)

FOOD	WEIGHT, GRAMS	APPROXIMATE POTENTIAL ACIDITY, CUBIC CENTIMETERS N ACID	APPROXIMATE POTENTIAL ALKALINITY, CUBIC CENTIMETERS N BASE
Apple sauce	100		2.0
Canned tomatoes	100		3.0
Lettuce	50		3.7
Potatoes	150		10.5
Canned peaches	100		3.0
Milk	300–0		7.11– 0
Lean beef	90–75	10.8– 9	
Eggs	90	9.9	
Cornflakes	20–0	2.0– 0	
Shredded wheat	0–27	0 – 3.3	
Bread (white)	125 (average)	8.0	
Vanilla wafers	40 (average)	2.5	
	Totals	33.2–32.7	29.3–22.4
Orange juice	928		41.66
Grape juice	800		31.2

Note. Where two figures are given those in the first column apply to diet A and those in the second column to diet B only. Calculations for butter, salad oil, and cream are omitted because figures for these could not be obtained.

The pH determinations were made colorimetrically, great care being taken to protect the samples from the air by covering with mineral oil, after carefully removing with a pipette from beneath the layer of toluene, with which the urine was kept covered at all times. Folin's method ('22) was used for the determination of titratable acidity; organic acids were titrated according to the method of Van Slyke and Palmer

('20) and, for citric acid, the pentabromacetone method, as modified by Amberg and McClure ('17) and McClure ('22), was employed. The results are given in tables 2 and 3.

The changes in urinary pH, titratable acidity and total organic acids produced by the orange juice and grape juice agree, in general, with results obtained by other workers.

There is a significant increase in citric acid excretion with both the orange juice and the grape juice. This increase is slightly greater with the orange juice, but the ratio of the amount excreted to the amount ingested is very much higher for the grape juice. With one subject, the increased excretion resulting from the grape juice actually exceeds by 20 per cent the amount of citric acid contained in the grape juice. It is evident from these results that citric acid must have a source other than that of the citric acid ingested.

The relation between the citric acid excreted and the total organic acids excreted is also of interest. There is no constancy in this relationship either on the basal diets or on the basal diets with the fruit juice supplements.

If the average citric acid increase for each subject for the 5-day period is compared with the average total organic acid increase produced by the orange juice, the former is approximately 40 to 60 per cent of the latter. With the grape juice, the citric acid increase represents 35 to 40 per cent of the total organic acid increase. It is evident, then, than organic acids other than citric account for a considerable portion of the increased organic acid output resulting from the ingestion of orange juice. Since the acid of orange juice appears (Hartman and Hillig, '30) to be practically 100 per cent citric acid the other acids must be formed in the utilization of the citric acid or have some other metabolic source. The increase in citric acid as compared with the increase in total organic acids produced by the grape juice is far too high to be explained on the basis of unutilized citric acid of the grape juice. It can only be accounted for as representing chiefly a metabolic product.

TABLE 2

Effect of orange juice on acid components of the urine

	DAY	VOLUME, CUBIC CENTIMETERS	pH	0.1 N TITRATABLE ACIDITY, CUBIC CENTIMETERS	0.1 N ORGANIC ACIDS CUBIC CENTIMETERS	CITRIC ACID		VOLUME, CUBIC CENTIMETERS	pH	0.1 N TITRATABLE ACIDITY, CUBIC CENTIMETERS	0.1 N ORGANIC ACIDS CUBIC CENTIMETERS	CITRIC ACID	
						Grams	Cubic centimeters 0.1 N acid					Grams	Cubic centimeters 0.1 N acid
		Subject B.						Subject S.					
Basal diet A	1 2 3	Adjustment period—no analyses.						Adjustment period—no analyses.					
	4	1390	5.8	264	556	....	..	1380	5.9	280	717	....	...
	5	1200	5.5	375	489	0.403	63	1400	6.0	269	806	0.664	104
	6	900	5.6	278	413	0.307	48	1080	5.7	287	496	0.427	67
	7	790	6.0	224	349	0.426	66	690	5.5	285	416	0.510	79
	8	420	5.4	325	368	0.310	48	515	5.4	323	406	0.474	74
		Aver.	5.6	293	435	0.361	56	Aver.	5.7	288	568	0.527	82
Basal diet A plus 1000 cc. orange juice	1 2 3	Adjustment period—no analyses.						Adjustment period—no analyses.					
	4	1940	6.5	148	626	0.853	133	1990	6.1	251	692	....	...
	5	1700	6.8	147	487	1.044	163	2920	6.5	215	685	2.102	330
	6	1640	6.8	153	681	1.017	159	2360	6.6	185	849	1.746	272
	7	1500	6.8	137	585	0.925	144	2430	6.0	201	699	1.749	272
	8	1410	6.8	147	663	0.937	146	2340	6.3	234	907	1.688	264
		Aver.	6.7	146	608	0.962	150	Aver.	6.3	217	746	1.821	284
		Subject R.						Subject K.					
Basal diet B	1 2 3	Adjustment period—no analyses.						Adjustment period—no analyses.					
	4	1500	5.4	240	444	0.463	72	1515	5.5	262	469	0.424	66
	5	1635	5.4	304	444	0.478	74	2230	5.9	238	474	0.570	89
	6	2350	5.5	363	605	0.796	124	1400	5.6	218	418	0.489	76
	7	1650	5.4	308	505	0.702	109	1400	5.6	298	408	0.325	51
	8	1825	6.3	198	563	0.815	127						
		Aver.	5.6	282	512	0.658	102	Aver.	5.6	254	442	0.462	70

	7	1650	5.4	308	505	0.702	109	1400	5.6	298	408	0.325	51
	8	1825	6.3	198	563	0.815	127						
		Aver.	5.6	282	512	0.658	102	Aver.	5.6	254	442	0.462	70
Basal diet B plus 1000 cc. orange juice	1 2 3	Adjustment period—no analyses.						Adjustment period—no analyses.					
	4	1830	6.7	190	609	0.929	145	1395	6.6	124	649	0.552	86
	5	2000	6.5	138	674	1.242	194	1420	6.6	186	513	0.681	106
	6	1590	6.3	240	604	1.381	216	1900	6.3	194	518	0.895	140
	7	1605	6.7	171	614	1.068	167	1519	6.6	159	475	0.617	96
	8	1720	6.0	241	626	0.975	152						
		Aver.	6.4	196	625	1.115	174	Aver.	6.5	165	536	0.686	107
		Subject M.H.						Subject C.S.					
Basal diet A	1 2 3	Adjustment period—no analyses.						Adjustment period—no analyses.					
	4	2050	6.2	232	508	....	...	1600	5.8	209	512	....	..
	5	1350	6.0	283	489	0.648	101	950	5.8	219	478	0.437	67
	6	1350	5.8	253	540	0.451	70	740	5.6	269	482	0.472	74
	7	1560	5.6	306	507	0.541	84	590	5.6	298	424	....	..
	8	720	5.4	267	430	0.748	115	420	5.6	212	436	0.537	84
		Aver.	5.8	268	495	0.597	93	Aver.	5.7	241	466	0.482	75
Basal diet A plus 1000 cc. orange juice	1 2 3	Adjustment period—no analyses.						Adjustment period—no analyses.					
	4	1560	6.5	139	586	....	...	1920	6.9	125	660	0.806	126
	5	1610	6.9	108	624	0.837	131	2010	6.8	178	503	0.924	144
	6	2080	6.8	153	648	1.027	160	2360	6.8	144	566	0.944	147
	7	1570	6.9	136	496	0.971	151	1780	6.8	139	552	0.925	144
	8	1790	6.9	132	684	0.996	155	1700	6.8	170	653	0.816	127
		Aver.	6.8	133	607	0.956	149	Aver.	6.8	151	586	0.883	138

TABLE 3

Effect of grape juice on acid components of urine

	Day	Volume, cubic centimeters	pH	0.1 N titratable acidity, cubic centimeters	0.1 N organic acids cubic centimeters	Citric acid		Volume, cubic centimeters	pH	0.1 N titratable acidity, cubic centimeters	0.1 N organic acids cubic centimeters	Citric acid	
						Grams	Cubic centimeters 0.1 N acid					Grams	Cubic centimeters 0.1 N acid
		Subject C.						Subject H.					
Basal diet B	1 2 3	Adjustment period—no analyses.						Adjustment period—no analyses.					
	4	1170	5.5	323	531	0.721	112	1500	5.4	246	454	0.623	97
	5	1790	5.5	294	494	0.755	121	2065	6.4	166	450	0.584	91
	6	1780	5.5	332	536	0.930	145	2080	6.1	201	453	0.671	105
	7	1695	5.9	282	528	1.033	161	1545	5.9	216	424	0.546	85
	8	1690	6.1	210	538	0.917	143	1645	6.3	160	452	0.748	117
		Aver.	5.7	288	525	0.871	134	Aver.	6.0	197	446	0.634	99
Basal diet B plus grape juice	1 2 3	Adjustment period—no analyses.						Adjustment period—no analyses.					
	4	1699	5.6	320	552	0.982	137	1920	6.8	175	556	1.001	156
	5	1820	6.3	202	621	1.164	182	1655	6.1	195	573	0.900	140
	6	1740	5.8	270	576	1.083	170	1435	6.1	207	524	0.866	135
	7	1665	6.3	218	611	0.980	153	1500	6.3	206	540	0.895	141
	8	1770	6.0	236	618	0.934	146	1705	6.3	196	548	0.886	138
		Aver.	6.0	258	595	1.019	159	Aver.	6.3	195	548	0.909	141

Note: The figures for pH titratable acidity and organic acids for subjects R., K., C. and H. are the results of analyses made by students in a problem course. They are used by courtesy of Mrs. Ruth Cowan Clouse, who conducted the course.

TABLE 4

Citric acid ingested and excreted as a result of adding orange juice and grape juice to the basal diet

SUBJECT	B.	S.	M.H.	C.S.	K.	R.	C.	H.
Average citric acid excretion on basal diet. Grams	0.361	0.527	0.597	0.482	0.452	0.658	0.871	0.634
Average citric acid excretion on basal diet + 1000 cc. orange juice. Grams	0.962	1.821	0.956	0.883	0.686	1.115		
Average citric acid excretion on basal diet + 1000 cc. grape juice. Grams							1.019	0.909
Average increase in citric acid excretion. Grams	0.601	1.294	0.359	0.401	0.234	0.457	0.148	0.275
Amount of citric acid ingested in 1000 cc. orange juice. Grams	7.95	7.95	7.95	7.95	8.51	8.51		
Amount of citric acid ingested in 1000 cc. grape juice. Grams							0.23[1]	0.23[1]
Increased excretion in terms of per cent of amount ingested	7.5	16.6	4.5	5.3	2.6	5.3	64	120

[1] Based on average figures reported in J. Assoc. Official Agr. Chem., 1930, 13, 99.

A very small part of the increase in total organic acids with both the orange juice and grape juice is due to uric acid according to the findings of Clouse previously cited. Determination of other acids concerned in this increase must await the development of new methods now being investigated for use in the study of urinary acidity.

SUMMARY

Two studies made with eight women subjects on the effect of orange juice and grape juice on the urinary excretion of citric acid are reported. The effect on urinary pH, titratable acidity, and excretion of total organic acids was also observed.

The following results were obtained when 1000 cc. orange juice or 1000 cc. grape juice were added to a basal diet.

1. The pH was increased and the titratable acidity was decreased. Organic acid excretion as a whole was increased. These results confirm those obtained by other workers.

2. Citric acid excretion was increased. The increase produced by the orange juice was slightly greater than that produced by the grape juice, but the ratio of the amount of citric acid excreted to the amount ingested was very much higher for the grape juice. With one subject, the increase represented 20 per cent more than the citric acid contained in the grape juice. The latter result indicates a metabolic source of citric acid.

3. The increase in citric acid with the orange juice represented only 40 to 60 per cent of the total organic acid increase. Other organic acids must have been formed in the body.

4. Thirty-five to 40 per cent of the total organic acid increase with the grape juice was due to citric acid. Only a very small part of this could have come from the citric acid of the grape juice, and therefore, most of it can be accounted for only on the basis of having a metabolic origin.

The organic acids representing products of metabolism may arise as a result of the alkalizing effect of the fruit juices.

The author wishes to express her appreciation of the interest and advice of Mrs. Ruth Cowan Clouse at whose suggestion this study was begun. The hearty cooperation of the young women serving as subjects is gratefully acknowledged.

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URINARY EXCRETION OF CITRIC ACID

II. EFFECT OF INGESTION OF CITRIC ACID, SODIUM CITRATE AND SODIUM BICARBONATE

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In a previous paper (Schuck, '34) the increased output of citric acid when orange juice and grape juice supplements were added to a basal diet was reported. As a result of this study it was decided to investigate the influence of pure crystalline citric acid, sodium citrate and sodium bicarbonate upon citric acid excretion in order to obtain further information on: 1) The effect of an increased citric acid intake. 2) The effect of increased alkalinity together with increased citrate ion ingestion. 3) The effect of merely increasing alkalinity.

At the time this study was begun there were with the exception of the work of Gonce and Templeton ('30) no published reports on the effect on citric acid excretion of feeding citric acid to humans. And so far as the author was able to determine, the effect of sodium citrate on citric acid excretion had not previously been studied with humans.

Gonce and Templeton ('30) investigated the citric acid excretion of four normal children 7 to 12 years of age. They fed a basal diet for 2 days, and then supplemented the basal diet with dehydrated citric acid for a period of 3 days. The citric acid was fed at a level of 4 gm. per 15 pounds of body weight, with a range of 11 to 13 gm. daily for the different children. They found that the citric acid supplement did not result in increased citric acid excretion.

A number of studies have been reported on the effect of the administration of sodium bicarbonate on total organic acid excretion and on the excretion of certain specific organic acids, including citric. Fanconi ('28) observed that the organic acid excretion on a vegetable diet, which was already high, could be further increased by the addition of sodium bicarbonate or the potassium salt of another organic acid. Goiffon ('25, '28) observed increased excretion of citric acid on doses of sodium bicarbonate of from 5 to 15 gm. McCleod and Knapp ('18) found that lactic acid output was increased by the ingestion of sodium bicarbonate. Fasold ('30) in a study on a 10-year-old boy, in which he determined total organic acids, volatile acids, hippuric and citric acid, found that the total organic acids, hippuric and citric acid, were all higher on a vegetable than on a meat diet. When he further increased the alkalinity of the urine by adding sodium bicarbonate to the vegetable diet, the latter constituents were further increased. Östberg ('30) at about the same time, reported increased citric acid elimination resulting from administration of sodium bicarbonate. He found that there was a close correlation between citric acid excretion and ammonia excretion. With a decrease in citric acid, there was an increase in ammonia, and vice versa. As a result, he concluded that citric acid plays at least a small part in acid-base balance regulation.

In a later study Östberg ('31) reports an investigation of the effect of citric acid, sodium citrate, sodium bicarbonate, hydrochloric acid, ammonium chloride, calcium chloride, and a low carbohydrate to fat ratio on citric acid excretion. He found that with citric acid doses of from 10 to 15 gm., there was greatly increased output in one case, and in another case a decrease. Thirty grams showed in one case an increase from 0.78 to 1.5 gm., and in another only a small increase. Thirty to 40 gm. gave for one subject in periods of 2 or 3 days a slightly decreased output, accompanied by a decrease in pH and increase in ammonia. This variability in effect led to the conclusion that in cases in which citric acid influences

the acid-base balance, its administration brings about a decreased output, and in cases where there is no effect on the acid-base balance, administration of citric acid may increase its excretion. Östberg ('31) found that increased alkalinity due to ingestion of either sodium citrate or sodium bicarbonate gave increased excretion of citric acid.

Boothby and Adams ('32) have confirmed Östberg's results with sodium bicarbonate, but they feel that the significance of citric acid as a factor in acid-base balance regulation is still questionable. They point out in this connection that it is possible to have an acid urine containing large amounts of organic acids with the normal amount of citric acid. Süllman and Shearer ('32) obtained an increase in citric acid excretion with both sodium carbonate and sodium citrate, but when both were administered at the same time the figure was not significantly different from that obtained when either was used alone.

EXPERIMENTAL

The subjects for this study were six young graduate women of the nutrition department of The University of Chicago. Four of these, B., M.H., S. and C.S., were the same as those serving in the study previously reported (Schuck, '34), and the basal diet for this group was diet A, the composition of which has already been given. The basal diet for the two new subjects, M. and V., which we shall designate diet C, consisted of the following: apple sauce, 100 gm.; cornflakes, 20 gm.; American cheese, 30 gm.; head lettuce, 100 gm.; canned tomatoes, 100 gm.; potatoes, 150 gm.; ground lean steak, 90 gm.; canned peaches, 100 gm.; whole milk, 1¼ cups; thin cream, ¼ cup; bread, butter and vanilla wafers in amounts to complete the caloric requirements of the different subjects.

The weights of the subjects were followed as before, and their constancy indicated that caloric needs were met throughout the experiment.

The supplements used were citric acids, 12 gm. daily, a chemically equivalent amount of sodium citrate, and sodium

bicarbonate, 10 gm. daily. The citric acid was administered to all six subjects, the sodium citrate to B., M.H., S. and C.S., and the sodium bicarbonate to C.S., M. and V. An 8-day period was allowed for each supplement, the first 3 serving as preliminary days, during which the subject might become adjusted to the new substance. Collections and analyses were made during the remaining 5 days of the period.

The supplements were dissolved in water and the daily amounts were consumed in three separate doses through the day. The citric acid was diluted and sweetened to make a palatable and pleasing beverage, but palatability could not be attained with the other supplements.

Urine collections and analyses were made as described in an earlier study (Schuck, '34). The results are given in tables 1 and 2.

It is apparent that the ingestion of citric acid had little or no effect on the pH of the urine. In most cases, there was a slight decrease in titratable acidity and a decrease in the total organic acid excretion. The citric acid output was decreased in some instances and increased in others. As can readily be seen, there was no parallelism between the total organic acid excretion, as measured by the Van Slyke Palmer method, and the citric acid excretion—a finding which is in agreement with observations made by Östberg ('31).

The sodium citrate gave in every case a marked increase in pH, a marked decrease in titratable acidity, and a large increase in the total organic acids and citric acid excretion. The total organic acids and citric acid amounted to two or three times the excretion on the basal diet. The effect on pH and citric acid excretion produced by the sodium citrate is in general agreement with the results obtained by Östberg ('31). He fed sodium citrate at lower levels, so that the change in pH was not so great, and correspondingly the increase in citric acid excretion was less.

The effect of the sodium bicarbonate on the pH, titratable acidity and citric acid was similar to that of the sodium citrate, in that there was a significant increase in the pH and

TABLE 1

Effect of citric acid and sodium citrate on acid components of the urine

	DAY	VOLUME, CUBIC CENTIMETERS	pH	0.1 N TITRATABLE ACIDITY, CUBIC CENTIMETERS	0.1 N ORGANIC ACIDS CUBIC CENTIMETERS	CITRIC ACID		VOLUME, CUBIC CENTIMETERS	pH	0.1 N TITRATABLE ACIDITY, CUBIC CENTIMETERS	0.1 N ORGANIC ACIDS CUBIC CENTIMETERS	CITRIC ACID	
						Grams	Cubic centimeters					Grams	Cubic centimeters
		Subject B.						Subject M.H.					
Basal diet A	1	Adjustment period—no analyses.						Adjustment period—no analyses.					
	2												
	3												
	4	1390	5.8	264	556	....	..	2050	6.2	232	508	....	...
	5	1200	5.5	375	489	0.403	63	1350	6.0	283	489	0.648	101
	6	900	5.6	278	413	0.307	48	1350	5.8	253	540	0.451	70
	7	790	6.0	224	349	0.426	65	1560	5.6	306	507	0.541	84
	8	420	5.4	325	368	0.310	48	720	5.4	267	430	0.748	117
		Aver.	5.6	293	435	0.361	56	Aver.	5.8	268	495	0.597	93
Basal diet + citric acid	1	Adjustment period—no analyses.						Adjustment period—no analyses.					
	2												
	3												
	4	1190	5.9	140	339	0.547	84	1920	6.0	242	443	0.576	90
	5	960	6.0	230	345	0.555	87	1670	6.3	189	467	0.481	75
	6	770	5.6	263	385	0.622	97	1650	6.5	86	470	0.462	72
	7	680	5.4	296	467	0.598	93	830	5.7	224	492	0.481	75
	8	560	5.7	192	348	0.560	87	1280	6.2	184	501	0.512	80
		Aver.	5.7	244	376	0.576	90	Aver.	6.1	181	474	0.502	78
Basal diet + Na citrate	1	Adjustment period—no analyses.						Adjustment period—no analyses.					
	2												
	3												
	4	950	8.3	41	1001	1.243	194	1940	8.2	21	682	1.058	165
	5	1280	8.4	45	1303	1.321	206	1690	8.5	44	1365	1.418	221
	6	1550	8.3	121	867	....	...	1370	8.4	125	1274	1.507	250
	7	1560	8.2	162	1009	1.094	171	1640	8.3	214	1567	1.501	234
	8	1540	8.2	194	1134	1.212	189	1600	8.2	150	1867	1.984	325
		Aver.	8.2	112	1262	1.217	190	Aver.	8.3	110	1357	1.489	231

TABLE 2

Effect of citric acid, sodium citrate and sodium bicarbonate on acid components of the urine

	DAY	VOLUME, CUBIC CENTIMETERS	pH	0.1 N TITRATABLE ACIDITY, CUBIC CENTIMETERS	0.1 N ORGANIC ACIDS CUBIC CENTIMETERS	CITRIC ACID		VOLUME, CUBIC CENTIMETERS	pH	0.1 N TITRATABLE ACIDITY, CUBIC CENTIMETERS	0.1 N ORGANIC ACIDS CUBIC CENTIMETERS	CITRIC ACID	
						Grams	Cubic centimeters					Grams	Cubic centimeters
		Subject S.						Subject C.S.					
Basal diet A	1 2 3	Adjustment period—no analyses.						Adjustment period—no analyses.					
	4	1380	5.9	280	717	....	...	1600	5.9	209	512	....	..
	5	1400	6.0	269	806	0.664	104	950	5.8	219	478	0.437	68
	6	1080	5.7	287	496	0.427	67	740	5.6	269	482	0.472	74
	7	690	5.5	285	416	0.510	79	590	5.6	298	424	....	..
	8	515	5.4	326	406	0.474	74	420	5.6	212	436	0.537	84
		Aver.	5.7	288	568	0.527	82	Aver.	5.7	241	446	0.482	75
Basal diet + citric acid	1 2 3	Adjustment period—no analyses.						Adjustment period—no analyses.					
	4	2000	5.7	288	400	0.760	119	2150	6.1	187	458	0.871	127
	5	1900	5.6	306	474	0.836	130	1460	5.9	213	420	0.753	117
	6	1340	5.6	344	573	1.018	160	1950	5.7	244	421	0.818	127
	7	1980	5.7	292	583	1.029	161	1180	5.7	226	500	0.778	121
	8	2300	5.4	361	753	1.204	188	1080	5.7	225	401	0.864	133
		Aver.	5.6	318	556	0.969	151	Aver.	5.8	219	440	0.806	126
Basal diet + Na citrate	1 2 3	Adjustment period—no analyses.						Adjustment period—no analyses.					
	4	2070	8.1	62	1275	2.260	353	1380	8.2	18	1137	1.460	228
	5	1450	8.3	55	1352	2.146	335	1050	8.4	32	1827	1.507	235
	6	2400	8.2	190	1017	2.640	412	1990	8.2	74	812	1.576	242
	7	2120	8.0	177	768	1.888	295	1760	8.2	188	999	1.535	240
	8	2300	8.1	240	1612	2.208	345	1470	8.3	205	1764	1.793	280
		Aver.	8.1	144	1204	2.424	379	Aver.	8.3	103	1307	1.574	246
Basal diet + $NaHCO_3$	1 2 3	Adjustment period—no analyses.						Adjustment period—no analyses.					
	4	2000	8.2	18	1000	1.560	244	1600	8.2	18	1000	1.560	244
	5	1600	8.3	18	1000	1.560	244	1200	8.3	18	1000	1.560	244
	6	2000	8.2	18	1000	1.560	244	1600	8.2	18	1000	1.560	244
	7	2000	8.2	18	1000	1.560	244	1600	8.2	18	1000	1.560	244
	8	2000	8.2	18	1000	1.560	244	1600	8.2	18	1000	1.560	244
		Aver.	8.2	18	1000	1.560	244	Aver.	8.2	18	1000	1.560	244

	8	2300	8.1	240	1612	2.208	345	1470	8.3	205	1764	1.793	280
		Aver.	8.1	144	1204	2.424	379	Aver.	8.3	103	1307	1.574	246
Basal diet + $NaHCO_3$	1	Adjustment period—no analyses.						Adjustment period—no analyses.					
	2												
	3												
	4							960	7.8	106	1034	1.234	193
	5							1850	7.8	103	576	1.461	226
	6							2340	7.8	111	376	1.380	215
	7							1250	8.2	20	270	0.767	120
	8							1810	8.2	65	660	1.309	204
								Aver.	7.9	81	581	1.230	192
		Subject M.						Subject V.					
Basal diet C	1	Adjustment period—no analyses.						Adjustment period—no analyses.					
	2												
	3												
	4	1320	5.4	315	460	0.871	136	3810	6.4	217	504	0.521	81
	5	1700	5.6	338	464	0.965	150	3200	6.0	293	450	0.732	114
	6	1290	5.6	306	394	0.864	135	4260	6.6	195	494	0.592	92
	7	1990	6.2	285	510	1.066	167	4130	6.4	199	582	1.110	173
	8	920	5.5	262	568	0.956	149	4130	6.4	183	560	0.689	107
		Aver.	5.6	301	479	0.944	147	Aver.	6.3	217	518	0.728	114
Basal diet C + citric acid	1	Adjustment period—no analyses.						Adjustment period—no analyses.					
	2												
	3												
	4	1700	5.5	250	416	0.850	132	3860	5.6	252	248	0.984	153
	5	2270	6.0	283	494	0.825	129	4880	6.4	191	358	0.685	107
	6	1230	5.5	362	478	0.934	146	5860	6.4	185	578	0.808	126
	7	750	5.1	350	374	0.733	111	4810	6.2	268	530	0.550	86
	8	1260	5.5	371	366	1.072	167	4200	6.5	184	556	0.835	130
		Aver.	5.5	323	425	0.882	136	Aver.	6.2	216	454	0.772	120
Basal + Na HCO_3	1	Adjustment period—no analyses.						Adjustment period—no analyses.					
	2												
	3												
	4	1680	7.3	93	376	1.065	166	6510	7.3	155	484	0.856	133
	5	2230	7.3	124	536	1.361	213	5440	7.4	194	500	1.115	174
	6	1310	7.3	85	314	0.750	117	6550	7.4	221	704	1.506	234
	7	1785	7.5	121	620	1.159	181	6170	7.4	159	460	0.734	114
	8	2030	7.5	93	588	1.193	186	5910	7.4	141	538	1.229	192
		Aver.	7.4	103	487	1.105	173	Aver.	7.4	174	537	1.088	177

citric acid excretion and a decrease in titratable acidity. The excretion of total organic acids did not show a significant change with two of the subjects. One subject, however, showed an appreciable increase in total organic acid excretion. This is in agreement with the observations of other workers (Fanconi, '28; Goiffon, '25; and Fasold, '30).

The difference in effect upon citric acid excretions of feeding citric acid and sodium citrate in chemically equivalent amounts might be explained on the ground that the former is more completely utilized by the body, and that therefore there exists much less likelihood of any of it being excreted when fed in large amounts. Attention should be called to the fact, as having some possible bearing on the question, that the subjects while on the sodium citrate supplement experienced a feeling of nausea, malaise, and in some instances there was a marked diarrhea. The fact, however, that there was little change in the pH of the urine with the administration of citric acid, but that with the sodium citrate there was a marked increase in pH is significant in light of the theory that increased alkalinity is accompanied by increased citric acid excretion. The increase in citric acid output produced by administration of sodium bicarbonate is further evidence for the belief that citric acid is, at least, one of the important organic acids concerned with the elimination of excess base. The smaller sodium bicarbonate dosage used in this study did not bring about as great an increase in pH as was obtained with the sodium citrate, and there was also a smaller increase in the citric acid output. The pH and the citric acid increase, however, closely approach that obtained with the sodium citrate, while the effect upon total organic acid output is considerably less. The meaning of this is not clear.

The results of this investigation give increased evidence for the theory that citric acid excretion is influenced principally by changes in alkalinity, and not by the citric acid intake. They appear to strengthen the view that citric acid is one of the organic acids functioning in acid-base balance regulation.

SUMMARY

A study made with six women subjects of the effect of citric acid, sodium citrate and sodium bicarbonate on the urinary excretions of citric acid is here reported. The effect on urinary pH, titratable acidity, and excretion of total organic acids was also observed.

The following results were obtained with chemically equivalent amounts of citric acid and sodium citrate:

1. The citric acid produced little or no change in the pH of the urine; the sodium citrate brought about a marked increase.

2. The titratable acidity was slightly decreased as a result of the ingestion of citric acid, while the sodium citrate brought about a marked decrease.

3. Total organic acid excretion was decreased by the citric acid, but greatly increased by the sodium citrate.

4. Citric acid excretion was decreased in some cases and increased in others as a result of the citric acid ingestion while the sodium citrate brought about a marked increase in every case. The results here are in agreement with those obtained by Östberg.

5. The total organic acids and citric acid excreted as a result of the ingestion of sodium citrate amounted to two to three times the excretion on the basal diet.

The increase in pH and the decrease in titratable acidity brought about when sodium bicarbonate was fed were accompanied by a small increase in total organic acids and a considerable increase in citric acid excretion. Increase in total organic acid excretion on ingestion of sodium bicarbonate has been noted in the past, and, more recently, like observations have been made on citric acid excretion.

Apparently, citric acid excretion is not dependent upon citric acid ingestion.

Increased evidence is presented here for the view that citric acid is one of the organic acids which plays a part in acid-base balance regulation.

The author wishes to acknowledge with gratitude the hearty co-operation of the young women serving as subjects.

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